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(54) Title: OVINE CYTOKINE GENES

COMPARISON OF GAMMA-INTERFERON AMINO ACID SEQUENCES

	S1								S10										S20	1										
Ovine	M	K	Y	T	S	Y	F	L	A	L	L	L	C	V	L	L	G	F	S	G	S	Y	G	Q	G	P	F	F
Bovine	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	Q	-	-
Human	-	-	-	-	-	-	I	-	-	F	Q	-	-	I	V	-	-	S	L	-	C	-	C	-	D	-	Y	V
Murine	-	N	A	-	H	C	I	-	-	-	Q	-	F	L	M	A	V	S	G	/	C	-	C	H	-	T	V	I

	10								20								30													
Ovine	K	E	I	E	N	L	K	E	Y	F	N	A	S	N	P	D	V	A	K	G	G	P	L	F	S	E	I	L
Bovine	R	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human	-	-	A	-	-	-	-	K	-	-	-	-	G	H	S	-	-	-	D	N	-	T	-	-	L	G	-	-
Murine	E	S	L	-	S	-	N	N	-	-	-	S	-	G	I	-	-	E	E	K	/	S	-	-	L	D	-	W

	40								50								60													
Ovine	K	N	W	K	E	E	S	D	K	K	I	I	Q	S	Q	I	V	S	F	Y	F	K	L	F	E	N	L	K
Bovine	-	-	-	-	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human	-	-	-	-	-	-	-	-	R	-	-	M	-	-	-	-	-	-	-	-	-	-	-	-	K	-	F	-
Murine	R	-	-	Q	K	D	G	-	M	-	-	L	-	-	-	-	I	-	-	-	L	R	-	-	-	V	-	-

	70								80								90													
Ovine	D	N	Q	V	I	Q	R	S	M	D	I	I	K	Q	D	M	F	Q	K	F	L	N	G	S	S	E	K	L
Bovine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human	-	D	-	S	-	-	K	-	V	E	T	-	-	E	-	-	N	V	-	-	F	-	S	N	K	K	-	R
Murine	-	-	-	A	-	S	N	N	I	S	V	-	E	S	H	L	I	T	T	-	F	S	N	-	K	A	-	K

	100										110										120									
Ovine	E	D	F	K	R	L	I	Q	I	P	V	D	D	L	Q	I	Q	R	K	A	I	N	E	L	I	K	V	M
Bovine	-	-	-	-	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human	D	-	-	E	K	-	T	N	Y	S	-	T	-	-	N	V	-	-	-	-	-	-	-	-	-	Q	-	-
Murine	D	A	-	M	S	I	A	K	F	E	-	N	N	P	-	V	-	-	Q	-	F	-	-	-	-	R	-	V

	130													140															146
Ovine	N	D	L	S	P	K	S	N	L	R	K	R	K	R	S	Q	N	L	F	R	/	G	R	R	A	S	(M/T)			
Bovine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	/	-	-	-	-	-	-			
Human	A	E	-	-	-	A	A	K	T	G	-	-	-	-	-	-	-	M	-	-	-	/	-	-	-	-	-	Q		
Murine	H	Q	-	L	-	E	-	S	-	-	-	-	-	-	-	R	C	W	-	G	V	-	K	-	L	-	Q			

(57) Abstract

The present invention relates to a nucleic acid molecule comprising a nucleotide sequence encoding, or complementary to a sequence encoding, an ovine cytokine-like molecule. The preferred cytokine-like molecules include recombinant IFN-γ, GM-CSF, IL-1, IL-2, IL-4, TNFα and TNFβ.

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OVINE CYTOKINE GENES

The present invention relates generally to ovine cytokine genes and to recombinant products therefrom.

Cytokines are the hormones of the immune system which control and determine the nature of the immune response (Balkwill and Burke, 1989). Interleukins (numbered 1 to 8) primarily effect the functional activity of the lymphocytes involved in specific cell-mediated and antibody responses. Colony stimulating factors regulate the maturation of precursor cells into macrophages, granulocytes, mast cells and lymphocytes which are involved in innate resistance to many pathogens (Metcalf, 1987). The interferons, in addition to their direct anti-viral action, stimulate antibody synthesis, the activity of natural killer cells and the antimicrobial activity of macrophages and neutrophils (Bielefeldt Ohmann et al., 1987). All these molecules have the potential to alter the disease resistance and immune responsiveness of animals to a wide variety of infectious diseases and vaccines.

Gamma interferon (IFN-γ) plays a central role in the regulation of immune responses and is one of the most powerful modulators of macrophage activation (Balkwill and Burke, 1989). The T helper subset can be divided into two cell types on the basis of the cytokines they produce. TH1 cells secrete interleukin 2 (IL-2) and IFN-γ but not interleukin 4 (IL-4), whereas the TH2 cells synthesise IL-4 but not IFN-γ or IL-2.

The human murine and bovine IFN-γ genes have been cloned and fully characterised. Although there is a reasonable degree of homology between the DNA sequence of these genes (47-63%), the different IFN-γ molecules are generally species specific in their actions. With the

availability of recombinant bovine IFN-γ it has been possible to accurately study the effects of this molecule on various bovine cells (reviewed in Bielefeldt Ohmann et al., 1987).

Another cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF), primarily stimulates the development of granulocyte and macrophage precursor cells, but can also stimulate erythroid, eosinophil and megakaryocyte development at higher concentrations (Metcalf, 1984). GM-CSF is released by a variety of cell types (macrophages, T cells, endothelial cells, dendritic cells, stromal cells and fibroblasts) but only in response to a stimulatory signal such as bacterial lipopolysaccharide (LPS), antigen or in response to cytokines such as interleukin 1 (IL-1).

In addition to its documented role in haemopoiesis, GM-CSF also affects the functional capacity of myeloid cells. It enhances neutrophil function, the cytotoxic activity of monocytes/macrophages and leukocyte recruitment to inflammatory sites. It has the ability to induce secondary cytokine factors such as tumour necrosis factor (TNF) and IL-1 from monocytes and macrophages and to potentiate the functional capacity of both antigen-presenting cells and the T cell response (reviewed in Monroy et al., 1990).

The human murine and bovine GM-CSF genes have been cloned and characterised and purified recombinant proteins are currently available. These recombinant proteins are generally species-specific in their actions, the exception being human GM-CSF which has some activity on bovine bone marrow cells. This species restriction will require the availability of purified or recombinant GM-CSF for each economically important species, including sheep, to examine both the therapeutic and adjuvant

potential of this factor.

Interleukin 1 (IL-1) is a cytokine involved in the regulation of the immune and inflammatory response (reviewed in Durum et al., 1985). It was initially found to be secreted by activated monocytes. Subsequently, many other cell types including keratinocytes, fibroblasts and endothelial cells also produce IL-1. Two biochemically distinct but functionally related IL-1 proteins have been cloned, namely IL-1α and IL-1β (Auron et al., 1984; Gray et al., 1986). Both human IL-1α and IL-1β bind to the same receptors with similar affinities (Dower et al., 1986). Although they have similar biological activities and bind to the same receptor, IL-1α and IL-1β share less than 30% amino acid homology.

IL-1 exerts a wide spectrum of activities including induction of T and B lymphocyte proliferation and lymphokine production (reviewed in Durum et al., 1985); it stimulates arachidonic acid metabolism resulting in prostaglandin production; inflammatory proteins including collagenases and plasminogen activators and acute phase protein production are also induced by IL-1. With the involvement of IL-1 in wound healing (Gahring et al., 1985) and adjuvanticity (Staruch and Wood, 1983), IL-1 could be an important immunopharmacological agent not only in humans but also in the economically crucial meat and livestock industry. Bovine IL-1 has been cloned (Leong et al., 1988; Maliszweski et al., 1988). Both recombinant IL-1 and IL-2 have been demonstrated to be effective adjuvants in bovine herpesvirus-1 immunised and challenged calves (Reddy et al., 1990; Reddy et al., 1989). Species preference for bovine IL-1 has been shown (Lederer et al., 1989) and further work on species preference can be elucidated with ovine IL-1β.

The tumour necrosis family of cytokines includes two proteins, namely TNF-α or cachectin and TNF-β or lymphotoxin. TNF-α is produced mainly by monocytes and/or macrophages and TNF-β by lymphoid cells and are encoded by single copy genes. There is 30% amino acid homology between TNF-α and TNF-β and both are induced by different signals.

The two proteins exert a wide spectrum of biological activities including inhibition of viral replication, cytotoxic or cytostatic effects on tumour cell lines, induction of differentiation of promyelocytic cell lines, and induction of HLA class II major histocompatibility complex antigens. Both proteins bind to the same receptor on the cell surface. TNF-α has been implicated in a variety of disease states including meningococcal septicemia, cerebral malaria, graft versus host disease, cancer cachexia and antimalarial activity. TNF-α has also been shown to enhance the toxicity of eosinophils to schistosomula in vitro supporting the concept that TNF may play a beneficial role in parasitic infections. TNF-β also probably plays a role in parasitic infections. For example, malaria infected red blood cells induce TNF-β production and TNF-β can activate macrophages to kill the schistosomula of Schistomsoma mansoni.

In view of the ability of the TNFs to modulate the immune response and protect the host against infectious agents, these molecules represent important cytokines to investigate at the molecular level.

The cytokine interleukin-4 (IL-4) has a proliferative effect on B cells, can induce expression of class II major histocompatibility complex antigens on resting B cells and can induce expression of the low affinity receptors for the Fc portion of IgE. In addition, IL-4 enhances the secretion and cell surface expression of IgE

and IgG1 and it appears to be required to generate and sustain in vivo IgE responses and acts by causing heavy chain switching to IgE.

Besides B lymphocytes, IL-4 has been shown to stimulate proliferation of T lymphocytes and thymocytes in the presence of phorbol esters. It acts on connective tissue-type mast cells, haematopoietic cells and enhances the antigen presenting ability of macrophages.

The main source of IL-4 is subset of helper T cells (Th2) which are clearly defined in murine T cell clones (Mosmann et al, 1986). The Th2 subset contains the most effective helper activity for B cells, a large part of which can be attributed to IL-4. It is predicted that Th2 cells play a central role in modulating humoral responses to different antigenic stimuli. Studies on immunity against parasites have suggested that in many cases, the humoral antibody response plays a protective role. IgE is involved in antiparasite immunity and so far, it appears that IL-4 may be the only cytokine that can induce high IgE levels.

Considering the diverse activities of the cytokines and, in many cases, their species specificity, there is a need to clone and express cytokine genes from specific livestock animals, such as sheep. In accordance with the present invention, ovine cytokine genes have been cloned and their nucleotide sequences determined thereby permitting the development of a range of adjuvants, immunopotentiators and other therapeutic compositions comprising recombinant ovine cytokines.

Accordingly, one aspect of the present invention relates to a nucleic acid molecule comprising a nucleotide sequence encoding, or complementary to a sequence encoding, an ovine cytokine or a functional homologue,

derivative or mutant thereof. Such molecules will be collectively referred to in the specification and claims as "ovine cytokine-like molecules".

In accordance with the present invention, by "nucleic acid molecule" is meant a single or double stranded sequence of ribonucleotides or deoxyribonucleotides which encode, or are complementary to a sequence which encodes, an ovine cytokine-like molecule. Although not wishing to limit the scope of the present invention to specific cytokines, the preferred cytokines are IFN-γ, GM-CSF, IL-1, IL-2 and IL-4 and TNFα and TNFβ. One skilled in the art, however, will immediately recognise the wide applicability of the present invention to a range of other cytokines.

In a preferred embodiment, the nucleic acid molecule is cDNA or a synthetic DNA sequence and even more preferably, the DNA sequence forms part of an expression vector.

Any number of expression vectors can be employed depending on whether expression is required in a eukaryotic or prokaryotic cell. Examples of eukaryotic cells contemplated herein include mammalian, yeast and insect cells and examples of prokaryotes include Escherichia coli, Bacillus sp. and Pseudomonas sp.

General techniques of cloning and expression of DNA can be found in Maniatis et al. (1982) and Sambrook et al. (1989). In the present case, the ovine IFN-γ, GM-CSF, IL-1, IL-2 and IL-4 and TNFα and TNFβ genes were cloned using PCR oligonucleotides based on bovine or human sequences. The effectiveness of this approach suggests that this technique will be useful in cloning a whole range of ovine cytokines.

The present invention extends to recombinant ovine cytokines such as recombinant IFN-γ, GM-CSF, IL-1, IL-2 and IL-4 and TNFα and TNFβ. By "recombinant cytokine" is meant a glycosylated or unglycosylated polypeptide molecule, with or without other associated molecules (eg. lipids) produced by recombinant means such as presence of a DNA molecule in an expression vector in the correct reading frame relative to a promoter and introducing the resultant recombinant expression vector into a suitable host and growing said host under conditions appropriate for expression and, if necessary, transportation of the recombinant protein or its derivative from said host and then purifying the recombinant molecule.

Given the recombinant cytokines contemplated by the present invention, it is within the scope to include homologues, derivatives or mutants thereof prepared by any number of means. Such homologues, derivatives or mutants of ovine cytokines include single or multiple amino acid substitutions, deletions and/or additions to the molecule. Conveniently, these are prepared by first making single or multiple nucleotide substitutions, deletions and/or additions to the nucleic acid molecule encoding the ovine cytokine. Alternatively, once the amino acid sequence is known, amino acids can be chemically added by established techniques and in any sequence required to give the desired mutant. All such homologues, derivatives and mutants are encompassed by the term "cytokine-like molecule" as used in the specification and claims herein.

The recombinant ovine cytokines contemplated herein will find particular application in the intensive livestock industries such as the live animal export trade, feed-lots and intensive rearing industries. Animals in close containment are subjected to greater environmental challenge with infectious diseases, particularly

respiratory infections and are more prone to the immunodepressive effects of stress leading to higher susceptibility to opportunistic pathogens.

According to this aspect of the present invention there is provided a method for the treatment and/or prophylaxis of a livestock animal exposed to or infected with a pathogenic organism comprising administering to said animal an immunoresponsive effective amount of an ovine cytokine-like molecule for a time and under conditions sufficient to maintain, stimulate or enhance the immunoresponsiveness of said animal. Examples of pathogenic organisms contemplated by this aspect of the present invention include Dichelobacter nodosus, Haemonchus contortus, Trichostrongylus colubriformis, Ostertagia circumcincta, Fasciola hepatica, Listeria monocytogenes, Chlamydia psittaci, Fly-strike, Toxoplasma gondii, Corynebacterium pseudotuberculosis and Taenia ovis.

Preferably, the cytokine-like molecule is a recombinant molecule and even more preferably is selected from one or more of IFN-γ, GM-CSF, IL-1, IL-2, IL-4, TNFα and/or TNFβ. Generally, the animal will be a sheep.

Another important application of the cytokines is as natural adjuvants for vaccines, particularly for subunit vaccines produced by recombinant DNA technology. Some of these molecules, particularly the interleukins, have already been shown to enhance the immune response of immunodepressed animals to antigens delivered by viral vectors or, when incorporated into the emulsion, to enhance the antibody response to inactivated vaccines. Advances in slow-release technology and the development of live apathogenic bacteria and viruses as delivery vectors for these molecules will ensure their cost-effectiveness in sheep and cattle. Accordingly, the

present invention extends to a method of enhancing and/or stimulating an immune response to one or more antigens in an animal, such as an immunodepressed animal, comprising administering to said animal an immunoresponsive effective amount of an ovine cytokine-like molecule. The present invention, therefore, further extends to adjuvant compositions comprising one or more ovine cytokine-like molecules mixed with or coupled to an antigen. Such compositions may also contain one or more carriers and/or diluents acceptable for veterinary use. The adjuvant composition may also comprise an adjuvant nucleic acid molecule comprising a first nucleic acid molecule encoding one or more ovine cytokine-like molecules inserted into a viral or bacterial expression vector with a second nucleic acid molecule encoding an antigen or antigenic epitope such that both the cytokine and antigen genes are expressed.

Preferably, the cytokine-like molecule is selected from one or more of IFN-γ, GM-CSF, IL-1, IL-2, IL-4, TNFα and/or TNFβ. Generally, the preferred animal is a sheep.

The present invention is further described by reference to the following non-limiting Figures and Examples.

In the Figures:

Figure 1 shows the nucleotide and amino acid sequence of ovine IFN-γ. Numbering refers to the amino acid sequence above and the DNA sequence below. The putative signal sequence runs from the first amino acid S1 to S20, where the putative cleavage point is marked with arrows. Numbering restarts from the first amino acid of the proposed mature IFN-γ molecule. Dashed lines mark the sequence of the oligonucleotide primers (IFN-N and IFN-C) used in the PCR reaction.

Figure 2 shows a comparison of IFN-γ amino acid sequences. Numbering above the line refers to the amino acids below. Amino acids numbered with an S are presumed to be part of the secretory signal which is cleaved from the mature IFN-γ. A dash indicates identity with the ovine sequence and a slash indicates that the sequence has no corresponding amino acid at this point.

Figure 3 shows the nucleotide and amino acid sequence of ovine GM-CSF. Numbering refers to the DNA sequence above and the amino acid sequence below. The putative signal sequence runs from the first amino acid S1 to S17, where the putative cleavage point is marked (*). Numbering restarts from the first amino acid of the proposed mature GM-CSF molecule. Dashed lines mark the sequence of the oligonucleotide primers (GM-N and GM-C) used in the PCR reaction.

Figure 4 shows a comparison of GM-CSF amino acid sequences. Numbering above the line refers to the amino acids below. Amino acids numbered with an S are presumed to be part of the secretory signal which is cleaved from the mature GM-CSF. A dash indicates identity with the ovine sequence, and a slash indicates that the sequence has no corresponding amino acid at this point.

Figure 5 shows the nucleotide and amino acid sequence of cDNA encoding ovine IL-2. Numbering refers to the nucleotide sequence above and amino acid sequence below.

Figure 6 shows a comparison of IL-2 amino acid sequences. The star represents the predicted amino acid terminus of mature IL-2.

Figure 7 shows the nucleotide sequence of ovine IL-1β cDNA.

Figure 8 shows a comparison of ovine, bovine, human and murine IL-1β amino acid sequences as deduced from their DNA sequences. Numbering is based on the ovine amino acid sequence. The predicted amino acid terminus of mature IL-1β is marked with an asterisk (*). A potential N-glycosylation site is as marked (+). The amino acid residue that is identical to the ovine sequence is indicated by (-). Where the amino acid is not present, it is denoted by (.).

Figure 9: Nucleotide and inferred amino acid sequence of ovine TNF-α cDNA. The first strand cDNA was synthesised from RNA isolated from alveolar macrophages stimulated by lipopolysaccharide for 4h. PCR was performed based on the human TNF-α cDNA. The primer sequences are: ATG AGC ACT GAA AGC ATG ATC CGG and CAG GGC AAT GAT CCC AAA GTA at the 5' and 3' end, respectively. The PCR conditions were 35 cycles at 94°C for 1 min, 50°C for 2 min and 72°C for 2 min.

Figure 10: Alignment of ovine, bovine, human, murine and rabbit amino acid sequences for TNF-α as deduced from their cDNA sequences. Numbering is based on the ovine amino acid sequence. The predicted amino acid terminus of the mature protein is marked with an asterisk (*). The amino acid residue that is identical to the ovine sequence is indicated by (-). Where the amino acid is absent, it is denoted by (.).

Figure 11: Nucleotide and inferred amino acid sequence of ovine TNF-β cDNA. The first strand was synthesized using RNA isolated from lymph nodes stimulated with ConA and PMA for 21h. The sequence of the primer used in the PCR are: ATG ACA CCA CCT GAA CGT CT and CTA CAG AGC GAA GGC TCC AAA GAA at the 5' and 3' end, respectively. The PCR conditions were 35 cycles at 94°C for 1 min, 50°C for 2 min and 72°C for 2 min. The amino acids in the brackets

are encoded by a second cDNA clone with the corresponding change in the nucleotide beneath it.

Figure 12: Alignment of ovine, human and murine amino acid sequences as deduced from their TNF-β cDNA sequences. Numbering is based on the ovine sequence. the first amino acid residue of the mature protein is marked by an asterisk (*). A potential N-glycosylation site is as marked (+). The amino acid residue that is identical to the ovine sequence is indicated by (-). Where the amino acid is absent, it is denoted by (.). The amino acids in brackets are derived from a second ovine cDNA clone.

Figure 13: Nucleotide and inferred amino acid sequence of ovine IL-4 cDNA. The first strand was synthesized using RNA isolated from mesenteric lymph node cells stimulated with PMA and calcium ionophore A23187 for 4 h. The primers used in the PCR are: T AGC TTC TCC TGA TAA ACT AAT TGC CTC and ATG AGT TAT AAA TAT ATA AAT A. The PCR conditions were 35 cycles at 94°C for 1 min, 50°C for 2 min and 72°C for 2 min.

Figure 14: Alignment of ovine, human, murine and rat IL-4 amino acid sequence as deduced from their cDNA sequences. Numbering is based on the ovine sequence. The predicted start of the mature protein is marked with an asterisk (*). The amino acid residue that is identical to the ovine sequence is indicated by a dash (-). Where the amino acid is absent, it is denoted by (.). The cysteine residues are typed in bold. The potential N-glycosylation site is marked by (+) and also typed in bold.

Figure 15: SDS-PAGE analysis of proteins from the purification of recombinant IL-1β. Lane 1: low molecular weight protein standards (Biorad), lane 2:

soluble fraction from parental pGEX-2T lysates, lane 3: insoluble fraction from parental pGEX-2T lysates, lane 4: purified glutathione-S-transferase, lane 5: soluble fraction from pGEX-2T.IL-1β lysates, lane 6: purified IL-1β, lane 7: IL-1β- GST fusion protein. The protein were electrophoresed on a 15% polyacrylamide gel and the gel stained with Coomassie Blue.

Figure 16: SDS-PAGE analysis of proteins from the purification of IL-2 by glutathione-agarose affinity chromatography. Lane 1: low molecular weight prestained protein standards (Biorad), lane 2: crude fraction of proteins solubilised in 6M guanidine hydrochloride, lane 3: purified IL-2-GST fusion protein.

Figure 17: SDS-PAGE analysis of proteins from the purification of the recombinant IFN-γ. Lane 1: GST, lane 2: GST-ovine IFN-γ fusion, lane 3: thrombin cleaved ovine IFN-γ, lane 4: recombinant bovine IFN-γ and lane 5: Bio-Rad low molecular weight markers. The proteins were electrophoresed on a 15% polyacrylamide gel and then stained with Coomassie Brilliant Blue R.

EXAMPLE 1

Materials and Methods

Preparation and stimulation of ovine T-cells

A 2 year old Merino sheep was euthanased and a popliteal and a caudal cervical lymph node collected. The nodes were sliced before being forced through a stainless steel sieve to produce a single cell suspension. Cells were washed twice in Dulbecco's modification of Eagle's medium (Flow Laboratories, Australia) supplemented with 20 mM HEPES, 9 mM sodium bicarbonate, 2 mM L-glutamine, 50 uM 2-mercaptoethanol, 100 IU/ml penicillin, 100 ug/ml streptomycin and 10% (v/v) heat-inactivated foetal bovine serum (Flow Laboratories, Australia). Lymphocytes were

counted and resuspended at 10^7 per ml in the above media containing appropriate mitogen before culture in 75 cm^2 tissue culture flasks (50 ml/flask) for 6 hours at 37°C. Cells were centrifuged (10 min at 500g) and quickly resuspended in phosphate buffered saline before snap freezing in liquid nitrogen prior to storage at -70°C.

Preparation and stimulation of ovine alveolar macrophages

A one month old Merino lamb was euthanased and the lungs removed aseptically. The lungs were lavaged with 250 ml of phosphate buffered saline (PBS; pH 7.3) containing 6 mM EDTA. Approximately 150 ml of this solution was then removed from the lungs via sterile plastic tubing connected to a 50 ml syringe and the collected cells pelleted by centrifugation (500g for 10 min). The cells were washed twice in Dulbecco's modification of Eagle's medium (Flow, Australia) supplemented with 20 mM HEPES, 9 mM sodium bicarbonate, 2 mM glutamine, 50 µM 2-mercaptoethanol, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% (v/v) heat-inactivated foetal bovine serum. The cells were resuspended in culture medium and viable cells enumerated by trypan blue exclusion. Cells were found to be >95% macrophages by microscopic examination. The macrophages were cultured for 4h at 37°C in 90 mm plastic tissue culture petri dishes (4 x 10^7 cells/dish) containing 12 ml of culture media and 20 µg/ml LPS (Sigma, USA). The adhered macrophages were washed with PBS before being scraped off the surface of the dish, and then lysed in guanidinium isothiocyanate (Sambrook et al., 1989).

Cloning of the ovine IFN-γ gene

Total cellular RNA was extracted from Concanavalin-A (7.5 µg/ml) stimulated lymphocytes by guanidinium lysis and CsCl gradient purification as per Maniatis et al. (1982). Reverse transcription of 250 ng of RNA was in 25 µl of Taq polymerase buffer as recommended by the manufacturer

(Cetus Corporation, USA) including 0.15% (v/v) Triton X-100 and deoxynucleotide triphosphates at 200 µM. Priming of cDNA synthesis was either by oligo dT priming or using the PCR primers IFN-N (ATGAAATATACAAGCTATTTCTTAGC) and IFN-C (GCTCTCCGGCCTCGAAAGAGATT) at a final concentration of 20 ng/µl. Ten units of reverse transcriptase (Pharmacca, Sweden) was added and the reaction continued for 20 min at 37°C. DNA amplification was performed by taking aliquots of the first strand synthesis and making them up to 40 µl in Taq polymerase buffer as described above, including the PCR primers. PCR reaction conditions were 35 cycles of 94°C for 1.0 min, 55°C for 2.0 min, and 72°C for 3.0 min. PCR products were phenol/chloroform extracted prior to treatment with T4 DNA polymerase and T4 polynucleotide kinase as per Maniatis et al. (1982). Following purification on agarose gels, DNA inserts were extracted with geneclean (Bio101, USA) ligated into the SmaI site of pUC118 and electroporated into Escherichia coli JM109.

Cloning of the ovine GM-CSF gene

Total cellular RNA from LPS stimulated alveolar macrophages was extracted by CsCl gradient purification (Sambrook et al., 1989). Reverse transcription of 20 µg of RNA was performed in Taq polymerase buffer containing 50 mM KCl, 10 mM Tris. Cl (pH 8.3) and 10 µg/ml BSA. Deoxynucleotide triphosphates (1mM), 2.5 mM $MgCl_2$, and 50 µg/ml oligo dT were added to prime cDNA synthesis. An 18U aliquot of reverse transcriptase (Pharmacia, Sweden) was added to a final volume of 20 µl and the reaction continued for 1 hour at 42°C. PCR amplification was performed by adding the primers GM-N (5' ATG TGG CTG CAG AAC CTG CTT CTC C 3') and GM-C (5' CTT CTG GGC TGG TTC CCA GCA GTC A 3') at a final molar concentration of 20pmol, 5U of Taq polymerase (Amplitaq, Cetus, USA) and buffer to a final volume of 80 µl. PCR reaction conditions were 35 cycles of 94°C for 1 minutes, 55°C for

2 minutes, and 72°C for 2 minutes. PCR products were chloroform extracted prior to analysis on agarose gels and Southern Blot hybridisation probing with an end-labelled oligonucleotide primer based on the human GM-CSF sequence in a region that is 90% homologous with the bovine gene (5' TCG CCT CCA ACC CCG GAA ACT TCC TGT GCA 3'). Following treatment with T_4 polynucleotide kinase (Pharmacia, Sweden), the PCR products were purified on low melting point agarose gels, phenol extracted, ligated into the SmaI site of pUC119 and electroporated into Escherichia coli JM109 (Sambrook et al., 1989).

Cloning of the ovine IL-2 gene

Total cellular RNA was extracted from the 24h ConA(7.5µg/ml) and phorbol-myristate acetate (7.5ng/ml) stimulated lymphocytes by guanidinium thiocyanate lysis and CsCl gradient purification as in Maniatis et al. (1982). Reverse transcription of 1µg of RNA was in Taq polymerase buffer containing 50mM KCl, 10mM Tris.Cl (pH8.3) and 10µg/ml BSA. Deoxynucleotide triphosphates (1mM), 2.5mM $MgCl_2$ and 50µg/ml oligo dT were added to prime cDNA synthesis. 18U of reverse transcriptase (Pharmacia, Sweden) was added to a final volume 20µl and the reaction continued for one hour at 42°C. PCR amplification was performed by adding the primers IL-2 N (5' ATG TAC AAG ATA CAA CTC TTG TCT T) and IL-2 C (5' GTC ATT GTT GAG TAG ATG CTT TGA C) at a final molar concentration of 20 pmol, 5U of Taq polymerase (Amplitaq, Cetus USA), and buffer to a final volume of 80µl. PCR reaction conditions were 35 cycles of 94°C for one minute, 55°C for two minutes and 72°C for two minutes. PCR products were chloroform extracted prior to treatment with T4 polynucleotide kinase (Pharmacia, Sweden). The PCR products were purified on low melting point agarose gels, phenol extracted, ligated into the SmaI site of pUC19 and M13mp18 and electroporated into Escherichia coli JM109 (Sambrook et al., 1989). Two clones were

sequenced by the dideoxy method. The sequencing was performed in both orientations.

Cloning of IL-1β cDNA

Total cellular RNA from LPS stimulated alveolar macrophages was extracted by CsCl gradient purification (Sambrook et al., 1989). First strand cDNA was synthesised using 10 units of avian myelobalstosis virus reverse transcriptase (Pharmacia, Sweden) in the presence of deoxynucleotide triphosphates (1mM), 2.5mM $MgCl_2$ and 50 µg/ml oligo dT. The PCR was performed using primers based on the bovine cDNA sequence at the 5' and 3' end. The primer sequences are as follows: ATG GCA ACC GTA CCT GAA and CTA GGG AGA GAG CCT TTC CAT T. The PCR conditions were 35 cycles of 94°C for 1 minute, 55°C for 2 minutes and 72°C for 2 minutes. The PCR products were analysed on a 1% (w/v) agarose gel, gel purified on low melting agarose, ligated into the SmaI site of the plasmid pUC19 and electroporated into Escherichia coli JM109.

DNA sequencing was performed on both strands using the T7 DNA polymerase sequence kit (Pharmacia, Sweden).

Cloning of ovine TNF genes

The ovine TNFs were cloned by polymerase chain reaction (PCR). RNA was isolated from alveolar macrophages stimulated with lipopolysaccharide (20ug/ml) for 4 h and lymph nodes stimulated with Concanavalin A (7.5ug/ml) and phorbol myristate acetate (7.5ng/ml) for 2h. The RNA was purified by CsCl ultracentrifugation. The complementary DNA was synthesized using avian myelobalstosis virus reverse transcriptase and PCR performed. The amplified DNA fragment was subcloned into plasmid pUC118. DNA sequencing was performed by the dideoxy termination method. Two clones were sequenced for both TNF-α and β.

Cloning of ovine IL-4 gene

Total cellular RNA was extracted from 4h phorbol myristate acetate (7.5ng/ml) and calcium ionophore A23187 (0.5ug/ml) stimulated mesenteric lymph nodes by guanidinium thiocyanate lysis and CsCl gradient purification (Maniatis et al, 1982). Reverse transcription of 1ug of RNA was performed in Taq polymerase buffer containing 50mM KCl, 10mM Tris. Cl(pH8.3) and 10ug/ml BSA. Deoxynucleotide triphosphates (1mM), 2.5mM $MgCl_2$ and 50ug/ml oligo dT were added to prime the first stand cDNA synthesis. An aliquot of 18U of avian myeloblastosis virus reverse transcriptase (Pharmacia, Sweden) was added to a final volume of 40ul and the reaction was carried out for 60min at 42°C. PCR amplification was performed using 10ul of the first strand reaction and primers were: GATTCCATGGGTCTCACCTCCCAACTGCTT and CGGTCGACCTAGCTCGAACACTTTGAATATTT. PCR conditions were 35 cycles of 94°C for one min, 55°C for two min and 72°C for 2 min. The PCR product was phenol extracted, genecleaned and digested with EcoRI and SalI. After a second geneclean procedure, the PCR amplified fragment was ligated to pUC 118 which was digested with EcoRI and SalI. The ligated DNA was electroporated into Escherichia coli strain JM109. Three clones were sequence by the dideoxy method.

PCR amplication was also performed using another 10ul aliquot of the first strand reaction and primers based on the 5' and 3' untranslated region of human IL-4 cDNA (Yokota et al, 1986). The sequence of the primers were: T AGC TTC TCC TGA TAA ACT AAT TGC CTC and ATC AGT TAT AAA TAT ATA AAT A. The PCR product was phenol extracted, genecleaned and incubated with T_4 polymerase for 15 mins at room temperature followed by kinasing with T_4 polynucleotide kinase. The PCR product was then ligated into the SmaI site of

pUC119 and electroporated into JM109. The colonies were patched and their DNA hybridised with the partial ovine IL-4 cDNA obtained using the primers based on the coding sequence of human IL-4. Four clones were sequenced by the dideoxy method.

EXAMPLE 2

Ovine γ-interferon gene

The nucleotide sequence and inferred amino acid sequence of a cDNA encoding ovine IFN-γ is shown in Figure 1. Overall, the inferred IFN-γ protein has a molecular weight of 19,150 dalton. The last amino acid, either methionine or threonine, is assumed by analogy with the bovine sequence.

There were three sites where variation occurred between the ovine IFN-γ clone sequence above and another clone, at bases 153 (C-T), 189 (T-C) and 318 (C-T). At base 318 variation from C to T in the second clone is consistent with the bovine sequence, whereas at the other two points the sequence of the clone shown in Figure 1 matches that of the bovine. In no case do these variations alter the amino acid sequence of the protein.

The ovine DNA sequence shows an overall homology of just under 97% with the bovine sequence, excluding the terminal sections of the gene where the PCR primers dictate that the sequence will conform to that of the bovine. The carboxy terminal oligonucleotide used for PCR priming did not include the codon for the last amino acid.

Figure 2 shows the alignment of the inferred amino acid sequence with that of the IFN-γ genes of other species. The difference between the ovine and bovine sequence is 6 out of 126 amino acids or around 5% (excluding the primer

area), with one variation within the first twenty amino acids which are a signal region (Gray and Goeddel, 1982, 1983).

EXAMPLE 3

Ovine GM-CSF gene

The PCR product (see Example 1) was cloned into pUC119, and the presence of inserts confirmed by agarose gel analysis and by Southern Blot hybridisation probing with an end-labelled oligonucleotide based on the human GM-CSF sequence. Two separate clones were selected for DNA sequencing, which was performed on both strands of each clone. The sequence and inferred amino acid sequence of the identical clones is shown in Figure 3. Overall, the inferred GM-CSF molecule has a molecular weight of 16,285. The DNA sequence shows an overall homology of just under 91% with the bovine sequence, excluding the terminal sections of the gene where the PCR primers dictate that the sequence will conform to that of the bovine.

Figure 4 shows the alignment of the inferred amino acid sequence with that of the GM-CSF genes of other species. The ovine and bovine sequences are 81% homologous (excluding the primer area), with no variations occurring in the first 17 amino acids which are a signal region. The cysteine residues are conserved throughout all the species, indicating similar secondary structures formed by disulphide bonding.

EXAMPLE 4

Ovine IL-2 gene

The nucleotide sequence and inferred amino acid sequence of a cDNA encoding ovine IL-2 is shown in Figure 5.

There were four (4) sites where there was a single base change in the bovine and the ovine IL-2 sequence resulting in no change in amino acid. These were at base 153 (T to C; proline 51); at base 192 (T to C; phenylalanine 64); at base 342 (C to A; isoleucine 114) and at base 396 (A to G; alanine 132).

There were five (5) sites where variation occurred between the bovine and ovine IL-2 sequence resulting in a change in amino acid. These were at base 187 (G to A) resulting in change of aspartic acid to asparagine in ovine; at base 196 (G to A) with change in amino acid valine to methionine; at base 274 (A to G) with change in amino acid asparagine to aspartic acid; at base 301 (C to A) resulting in change of amino acid proline to threonine and at base 405 (C to G) with change in amino acid asparagine to lysine.

The ovine DNA sequence shows an overall homology of 98% with the bovine sequence.

Figure 6 shows the alignment of the inferred amino acid sequence with that of the IL-2 gene of other species. The ovine and bovine sequences are 97% homologous. The first 20 amino acids which serves as a signal sequence is well conserved in both bovine and ovine species. The star represents the predicted amino terminus of mature IL-2. The one potential N-linked glycosylation site in both bovine and ovine is present at position Asn70. This is absent in the human and murine homologs. The

positions of the three cysteines are conserved. Ovine IL-2, like the bovine and human homolog, lacks the unusual stretch of 12 glutamine residues that are present in the murine IL-2.

EXAMPLE 5

Ovine IL-1β gene

Figure 7 shows the nucleotide sequence for the ovine IL-1β cDNA. The numbering begins with initiator Met codon ending with a termination codon at nucleotide 801. It codes for 266 amino acids with a predicted relative molecular mass of 31,051. The ovine nucleotide sequence shows an overall homology of approximately 95% with that of the bovine sequence.

Figure 8 shows the alignment of the deduced amino acid sequence of the various species. Fourteen amino acid residues in the ovine sequence differed from the bovine sequence. These are at amino acid position 11 (Met→Val), 25 (Ala→Val), 27 (Asp→Gly), 35 (Isoleu→Thr), 56 and 110 (Phe→Leu), 84 (Asn→Arg), 115 (Pro→Ala), 134 (Ala→Asp), 145 (Leu→Pro), 150 (Asn→Ser), 176 (Lys→Arg), 243 (Arg→Glu) and 249 (His→Arg).

Previous studies have indicated that IL-1s are synthesized as precursor proteins (Auron et al., 1985) and post-translational processing removes approximately 110 residues from the amino terminus. Based on the alignment of bovine, human and murine sequences, the amino terminus of mature ovine IL-1β is likely to begin at Ala 114 (indicated by an asterisk). The putative N-glycosylation signal (Asn-X-Ser/Thr) is found at position 58 (marked as +).

The ovine IL-1β shares approximately 95% homology with the bovine sequence both at the nucleotide and amino acid level. The six cysteine residues are well conserved.

Other residues such as Phe at positions 155, 211, 225, 259 and Pro at 170, 191, 200 and 231 that may contribute to the secondary or tertiary structure of the protein are also conserved. Interestingly, at the amino terminal of the mature protein at position 115, the amino acid was alanine in the ovine protein unlike proline in other species. Both alanine and proline have non-polar side groups. Previous studies have shown that deletion of the amino terminal amino acid beyond position 117 (numbering according to ovine IL-1β) of human IL-1β resulted in loss of receptor binding and biological activity (Mosley et al, 1987). This suggests that the change of proline to alanine may not alter biological activity.

With the availability of the cDNA clone, studies on the structure/function relationship will allow the active sites to be determined, leading to the development of IL-1 agonists and antagonists.

It has been shown that bovine thymocytes respond preferentially to bovine IL-1 and bovine fibroblast proliferate in the presence of bovine IL-1 but not human or murine IL-1α (Lederer et al., 1989). It would be interesting to examine whether the species specificity also occur in the case of ovine cell types. In addition, it has been observed that the subpyrogenic doses of IL-1 which act as an adjuvant (Staruch and Wood, 1983) is of several orders of magnitude more effective than human IL-1 in the activation of bovine thymocytes. If this holds true for the ovine systems, the therapeutic dose of ovine IL-1 would be expected to be more immunogenic for sheep compared to IL-1 from other species. It is therefore

crucial to use ovine gene products for therapeutic applications in the sheep livestock and meat industry.

EXAMPLE 6

Ovine TNF genes

Figure 9 shows the nucleotide sequence and the inferred amino acid sequence of the cDNA for ovine TNF-α. The sequence includes an open reading frame of 233 amino acids encoding a protein of 25.4 kDa. The sequences of the two clones were identical.

Figure 10 shows the alignment of the deduced amino acid sequence of ovine TNF-α with the bovine, human, murine and rabbit homologs. The levels of homologies are 91, 88, 72 and 72%, respectively, excluding the sequences of the primers used in the PCR. The start of the mature protein is marked with an asterisk.

Mature ovine TNF-α consists of 157 amino acids (157 in human and 156 in bovine, murine and rabbit). There are no potential N-linked glycosylation sites in the ovine, bovine, human and rabbit TNF-α. Only mouse TNF-α is N-linked glycosylated. There are 2 cysteine residues at amino acid 145 and 177 and they are conserved in all 5 species. There is 1 methionine residue in ovine and bovine TNF-α at position 113 and none in the other species. Site-directed mutagenesis studies suggest that the receptor binding sites for this ligand resides at Ala 160, Ser 162 and Val 167. These residues are well conserved in all the species.

Figure 11 shows the nucleotide sequence of TNF-β and the inferred amino acid sequence. The sequence includes an open reading frame of 205 amino acids encoding a protein of 22.2kDa. Comparison of the nucleotide sequence of the two clones reveals three nucleotide differences at positions 31, 153 and 410 resulting in changes in the

amino acids. These are Arg to Gly, Gln to His and Leu to Pro at amino acid residue numbers 11, 51 and 137, respectively.

Figure 12 shows the alignment of the inferred amino acid sequence with that of the TNF-β cDNAs of other species. The signal sequence of 34 amino acids is highly conserved. Based on homology with the human and murine homologs, the predicted start of the mature protein is at Leu 35 (as marked by an asterisk). The mature protein consists of 171 amino acids and has a molecular weight of 17.6 kDa. The possible N-linked glycosylation site is at Asn 96 as marked (+) and this is well conserved in all 3 species. There is one cysteine residue in the ovine TNF-β molecule at position 120 similar to the murine homolog. There are no cysteine residues in human TNF-β and no methionine residues in ovine TNF-β. There are, however, 3 methionine residues in human TNF-β (at positions 54, 154 and 167) and one in the murine form at position 167. Interestingly, at amino acid residue number 11, the two clones have a different amino acid: it is either Arg as in the human homolog or Gly as in the murine form. At amino acid 51, the His residue in one of the ovine clones is also encoded by the human homolog. Leu 137 was conserved in one of the clones. However, in the second clone the single nucleotide difference resulted in the change from Leu to Pro. The ovine TNF-β sequence shares approximately 72 and 75% homology with the human and murine forms, respectively.

The striking difference between the TNF-α and β sequences is in the amino terminus of the precursor form. The 34 amino acid presequence of TNF-β shows characteristics typical of a signal peptide. In contrast, TNF-α has a 76 residue long precursor sequence (78 in the murine and rabbit). There is evidence that human TNF-α exists as a membrane-bound form and the long presequence serves to

anchor the precursor protein in the membrane. It is possible that the membrane anchored TNF-α at the cell surface mediates many of its immunomodulatory functions. Metabolic labelling studies show that TNF-β is rapidly secreted and not stored intracellularly. These studies have yet to be performed with the ovine system.

With the availability of the TNF cDNAs, mRNA production can be directly analysed at the site of a particular lesion in various infectious diseases of the sheep. The cDNAs can also be used as markers to study the genetic predisposition to various infection. Structure/function relationship studies can be studied with the proteins produced from the cDNA clones, leading to the development of agonists and antagonists.

EXAMPLE 7

Ovine IL-4 gene

Figure 13 shows the nucleotide and inferred amino acid sequence of ovine IL-4 cDNA. The cDNA insert is 536 base pairs long. There is a single open reading frame, with the first ATG codon located at nucleotide 59 ending with the termination codon TAG at nucleotide 466-468. Comparison with the human and murine cDNA showed that the level of homology was 65.9% and 51.5% respectively. Interestingly, a single nucleotide change from a C in the human cDNA to A at position 467, resulted in a stop codon. Thus, the ovine IL-4 did not have the last two Ser residues unlike the human homolog. The amino terminal portion of the predicted polypeptide is hydrophobic, characteristic of a signal peptide sequence.

Figure 14 shows the alignment of the amino acid sequence of IL-4 of various species as deduced from their cDNA sequence. The start of the mature protein for human and murine IL-4 is at His 23 and His 21 based on the N-terminal amino acid sequence of the secreted protein

(Paul and Ohara, 1987). In the case of rat IL-4, the predicted start of the mature protein is at His 23 based on the consensus sequences for signal peptides (McKnight et al, 1991). Based on homology, the inventors herein predict that the start of the ovine protein is at His 23 (as marked with an asterisk).

The mature ovine, human, murine and rat IL-4 are glycoproteins of 113, 129, 120 and 123 amino acid residues long, respectively. One position of potential N-glycosylation site at amino acid residue 62 is conserved in all the four species (as marked by +). Human IL-4 has an additional N-glycosylation site. In the case of murine and rat IL-4, there are 2 and 3 additional N-glycosylation sites, respectively.

The cysteine residues at position 17, 27, 48, 70 and 85 (numbering based on the ovine sequence) are well-conserved in all the four species. In mature ovine IL-4, there are two more cysteine residues at position 105 and 135 (the latter also found in human IL-4). In the signal peptide region, there are two cysteines at position 17 (conserved in all four species) and at position 13 which is found only in the ovine and rat sequence.

In summary, mature ovine, human and murine IL-4 has 6 cysteines and rat IL-4 has 7 cysteines. At amino acid level, ovine IL-4 shares 57% and 36.4% homology with human and murine IL-4, respectively.

EXAMPLE 8

Expression of ovine IL-1β gene

Construction of expression vector

The ovine IL-1β cDNA encoding the mature IL-1β protein was obtained by polymerase chain reaction using the following primers: GGATCC GCA GCC GTG CAG TCA and

CCGGTCGAC TAG GGA GAG AGG GTT TCC ATT C. The primers were synthesised with a cohesive BamH1 5' end and a blunt HincII 3' end. The amplified fragment was subcloned into the Sma1 site of pUC119 and DNA sequencing was performed. A clone with the identical sequence to the original clone was selected for insertion into the BamH1 and Sma1 sites of the expression vector pGEX-2T (Smith and Johnson, 1988). Transformants of E. coli strain JM109 were produced. The recombinant plasmid was designated pGEX-2T.IL-1β.

Expression and affinity purification of recombinant proteins

Overnight cultures of E. coli transformed with the parental or recombinant pGEX-2T plasmids were diluted 1:50 in 250ml of Luria Broth with 100ug/ml ampicillin. The cultures were grown for 2h at 37°C before adding IPTG (isopropyl-β-thiogalactopyranoside) to a concentration of 0.2mM. After 2h, the cultures were harvested and centrifuged and the pellets resuspended in 5ml of phosphate-buffered saline (PBS). The cells were lysed on ice by sonication and then centrifuged. The supernatant was loaded onto a 5ml glutathione-agarose bead column (sulphur-linkage, Sigma). The flow through was kept and the column was washed thoroughly with at least 5 bed volumes of PBS. The recombinant protein was eluted either as a fusion product with 5mM glutathione or as free form by cleavage with thrombin (1U) at 37°C for 1h. The eluted proteins were analysed on a 15% (w/v) SDS-polyacrylamide electrophoresis gel (Laemmli, 1970) and the gel was stained by 0.05% (w/v) Coomassie Brilliant Blue R.

Assay of Ovine IL-1β

IL-1β was assayed by its ability to stimulate growth of ovine thymocytes in the presence of sub-mitogenic concentrations of lectins.

The thymus was asceptically collected from a euthanised sheep. The thymus was then cut into small pieces before being passed through a stainless steel sieve to produce a single cell suspension. The thymocytes were suspended in Dulbecco's modificaton of Eagle's medium (DMEM; Flow Laboratories, Australia) supplemented with 20mM HEPES, 9mM sodium bicarbonate, 2mM L-glutamine, 100IU/ml penicillin, 100ug/ml streptomycin and 10% heat-inactivated foetal bovine serum (FBS; Flow Laboratories). After three washes in DMEM, thymocytes were resuspended in DMEM and viable cells counted by trypan blue exclusion. The assay was performed in 96-well tissue culture plates with 7 x 10^5 thymocytes per well in a total volume of 200 ul per well containing 2 ug/ml of the lectin PHA and serial dilutions of IL-1β. Cells were incubated at 37°C, in a humidified atmosphere of 5% CO_2 in air, for 72 hours before the addition of ^3H thymidine (0.5 uCi/well). Cells were harvested 16 hours later, using an automated cell harvester and the amount of ^3H thymidine incorporation determined by counting in a β radiation counter.

Induction of the tac promoter in the expression plasmid pGEX-2T.IL-1β resulted in a high level of expression of a fusion protein of approximately 44 kDa as revealed by SDS-PAGE analysis (Figure 15, lane 5). The recombinant protein can be recovered in the soluble fraction and the level of expression was approximately 25% of total accumulated proteins. Affinity chromatography of the soluble fraction on glutathione-agarose beads yielded the free form of mature recombinant ovine IL-1β with a molecular weight of approx. 18kDa (Figure 15, lane 6). Elution with glutathione yielded a fusion protein with a molecular weight of 44kDa consisting of GST linked to IL-1β (Figure 15, lane 7). The parental pGEX-2T expressed a 26kDa glutathione-S-transferase protein (Figure 15, lane 2) and this 26kDa protein was purified to

homogeneity (Figure 15, lane 4) by affinity purification on glutathione-agarose beads.

The recombinant ovine IL-1β product was shown to be biologically active both as a fusion protein coupled to GST and as a cleaved product (Table 1).

Table 1

Assay of Ovine Recombinant IL-1β

SAMPLE	CONCENTRATION (ng/ml) 4000	800	160	32	6.4	1.3
Pure GST	43[1] (5)[2]	156 (66)	326 (133)	200 (102)	242 (52)	236 (93)
GST fused IL-1β	506 (103)	1102 (82)	691 (51)	450 (92)	239 (63)	145 (32)
Cleaved IL-1β	1209 (268)	1637 (122)	1018 (77)	611 (39)	320 (169)	329 (69)
Rec Hu IL-1β	146 (50)	903 (96)	600 (166)	525 (207)	564 (277)	332 (132)

[1] All values are CPM's and are the average of triplicates. Negative control 151 (30)

[2] Values in parentheses are standard deviations.

EXAMPLE 9

Expression of Ovine Interleukin-2 gene

Construction of expression vector

The ovine IL-2 cDNA encoding the mature IL-2 protein was obtained by polymerase chain reaction using the following primers: CCGGATCCGCA CCT ACT TCA AGC TCT and CCGGAATTC TCA AGT CAT TGT TGA GTA. The primers were synthesized with a cohesive BamH1 5' end and EcoRI 3' end. The amplified fragment was ligated into the BamH1 and EcoRI site of the expression vector pGEX-2T (Smith and Johnson, 1988). Transformants of E. coli strain JM109 were produced. The recombinant plasmid was designated pGEX-2T.IL-2.

Expression of affinity purification of recombinant proteins:

Overnight cultures of E. coli transformed with the parental or recombinant pGEX-2T plasmids were diluted 1:50 in 250ml of Luria Broth with 100ug/ml ampicillin. The cultures were grown for 2h at 37°C before adding IPTG (isopropyl-β-thiogalactopyranoside) to a concentration of 0.2mM. After 2h, the cultures were harvested and centrifuged and the pellets resuspended in 5ml of phosphate-buffered saline (PBS). The cells were lysed on ice by sonication and then centrifuged. An aliquot of the supernatant (10ul) was analysed on a SDS-polyacrylamide gel. The pellet was resuspended in 2.5ml of 6M guanidinium chloride to rescue the recombinant proteins from the inclusion bodies. After centrifugation, the supernatant was diluted two fold and applied onto a glutathione-agarose (sulphur-linkage, Sigma) column. The flow through was kept and the column was washed thoroughly with at least 5 bed volumes of PBS. The recombinant protein was eluted either as a fusion product with 5mM glutathione or as free form by cleavage

with thrombin (1U) at 37°C for 1h. The eluted proteins were analysed on a 15% (w/v) SDS-polyacrylamide gel electrophoresis (Laemmli, 1970) and the gel was stained by 0.05% Coomassie Brilliant Blue R.

Assay of Ovine IL-2

Recombinant IL-2 was assayed by measuring its ability to maintain the proliferation of Concanavalin A (Con A) T-lymphocyte blasts.

Ovine peripheral blood was collected into sodium citrate (final concentration of 0.38%). The blood was then centrifuged at 800g for 20 min, the buffy coat removed, diluted 1/2 in Alsever's solution and overlayed onto an equal volume of Lymphopaque (BDH; 1.086 g/ml). After centrifugation at 800g for 25 min, peripheral blood lymphocytes (PBL) were collected from the interface and washed twice in Alsever's solution (450g for 10 min) before a final wash in DMEM. PBL were resuspended in DMEM, viable cells enumerated by trypan blue exlcusion, and the concentration adjusted to 5×10^6 per ml. PBL were cultured with 7.5 ug/ml of Con A for 4-5 days at 37°C, in a humidified atmosphere of 5% CO_2 in air, in $75cm^2$ tissue culture flasks.

Con A blast cells were isolated by centrifugation over Lymphopaque (800g for 15 min), washed twice in DMEM and counted by trypan blue exclusion. The assay was performed in 96-well tissue culture plates with 5×10^4 Con A blasts per well in a total volume of 200 ul per well, containing serial dilutions of IL-2 samples. Cells were incubated at 37°C, in a humidified atmosphere of 5% CO_2 in air, for 24 hours before the addition of ^3H thymidine (0.5 uCi/well). Cells were harvested 16 hours later, using an automated cell harvester, and the amount of ^3H thymidine incorporation determined by counting in a β radiation counter.

Expression

Inducation of the tac promoter in the expression plasmid pGEX-2T.IL-2 resulted in a high level of expression of a fusion protein of approximately 42kDa as revealed by SDS-PAGE analysis (Fig. 16, lane 2). The recombinant protein cannot be recovered in the soluble fraction. Affinity chromatography of the proteins solubilised in 6M guanidinium chloride, on glutathione-agarose beads was performed. Elution with glutathione yielded the fusion protein of molecular weight of 44kDa consisting of GST linked to IL-2 (Fig. 16, lane 3). The parental pGEX-2T expressed the 26kDa glutathione-S-transferase protein (Fig. 15, lane 2) and this 26kDa protein was purified to homogeneity (Fig. 15, lane 4) by affinity purification on glutathione-agarose beads.

The recombinant IL-2 GST fusion product was shown to be biologically active when examined for its ability to maintain the growth of T cell blasts (Table 2).

Table 2

Assay of Recombinant Ovine IL-2

CONCENTRATION (ng/ml)	CPM		
	Pure GST	GST fused IL-2	rHuIL-2
10000	357 (143)	9283 (717)	9896[1](748)
5000	533 (64)	9319 (115)	9284 (743)
2500	857 (91)	9311 (685)	7978 (558)
1250	1074 (37)	8727 (547)	5943 (770)
625	1430 (115)	7417 (367)	4342 (402)
312	1428 (75)	6078 (676)	2755 (307)
156	1533 (133)	4856 (341)	2373 (172)
78	1482 (172)	3793 (580)	1942 (234)
39	1475 (144)	2883 (268)	1790 (116)
20	1466 (160)	2481 (282)	1745 (204)
10	1564 (126)	2095 (35)	1780 (79)
5	1536 (128)	1965 (85)	1770 (75)

[1] rHuIL-2 20 Units/ml titrated 1/2

Negative control 1691 (123)

All CPM's are the average of triplicates.

Values in parenthesis are standard deviations.

EXAMPLE 10

Expression of Ovine IFN-γ in Escherichia coli

Construction of expression plasmid

The ovine IFN-γ gene which had been cloned into the vector pUC118 (Radford et al, 1991) was used for sublconing into the pGEX expression system (Smith and Johnson, 1988). The sequence coding for the mature protein was excised from the pUC118 construct by MscI/EcoRI digestion and was ligated with SmaI/EcoRI

digested pGEX-2T. The ligated plasmids were transformed into E. coli JM109 by electroporation using a Bio-Rad Gene pluser according to manufacturer's instructions. The clone was named pGEX-2T.IFN.

Immunoblotting

Transformants were screened for expression of IFN-γ by immunoblotting bacterial colonies (Kemp et al, 1983) using monoclonal antibodies (Wood et al, 1990b) raised against recombinant bovine IFN-γ.

Expression of affinity purification of recombinant proteins

Overnight cultures of E. coli transformed with the parental or recombinant pGEX-2T plasmids were diluted 1:50 in 250ml of Luria Broth with 100ug/ml ampicillin. The cultures were grown for 2h at 37°C before adding IPTG (isopropyl-β-thiogalactopyranoside) to a concentration of 0.2mM. After 2h, the cultures were harvested and centrifuged and the pellets resuspended in 5ml of phosphate-buffered saline (PBS). The cells were lysed on ice by sonication and then centrifuged. An aliquot of the supernatant (10ul) was analysed on a SDS-polyacrylamide gel. The supernatant was applied onto a glutathione-agarose (sulphur-linkage, Sigma) column. The flow through was kept and the column was washed thoroughly with at least 5 bed volumes of PBS. The recombinant protein was eluted either as a fusion product with 5mM glutathione or as free form by cleavage with thrombin (1U) at 37°C for 1h. The eluted proteins were analysed on a 15% (w/v) SDS-polyacrylamide gel electrophoresis (Laemmli, 1970) and the gel was stained by 0.05% Coomassie Brilliant Blue R.

Assay of ovine IFN-γ

Recombinant ovine IFN-γ was assayed in both a bio-assay and an enzyme immuno-assay (EIA) which is specific for ovine, bovine and caprine IFN-γ. The bio-assay has been previously described by Wood et al. (1990a) and the EIA reported by Rothel et al (1990).

Expression

Induction of the tac promoter of the expression plasmid pGEX-2T.IFN resulted in a high level of expression of a soluble fusion protein of approximately 40kDa as estimated by SDS-PAGE (Fig. 17, lane 2). Affinity chromotography of the soluble fraction of sonicated cells yielded pure recombinant protein. Thrombin cleavage of the fusion resulted in the separation of IFN-γ from the GST moiety (Fig. 17, lane 3).

The biological activity (Units/mg) of the recombinant ovine IFN-γ was estimated in the bovine IFN-γ EIA using recombinant bovine IFN-γ (Ciba-Geigy Ltd. Lot No. AE62) of known activity as the reference standard. The results are shown in Table 3.

Table 3

Activity of Recombinant Ovine IFN-γ in EIA

Sample	Specific Biological Activity[1] (Units/mg)
Recombinant Bovine IFN-γ[1]	2.5×10^6
GST fused ovine IFN-γ	3.1×10^5
Cleaved ovine IFN-γ	4.8×10^5
GST	not detected

[1] Used as reference standard.

Both cleaved and GST-fused ovine IFN-γ protected bovine kidney cells from challenge with Semliki Forest virus in the IFN bioassay (Table 4).

Table 4

Activity of Recombinant Ovine IFN-γ in Bio-Assay

SAMPLE	SPECIFIC BIOLOGICAL ACTIVITY (U/mg)
Recombinant Bovine IFN-γ[1]	$>2.4 \times 10^7$
GST fused ovine IFN-γ	3.2×10^5
cleaved ovine IFN-γ	2.6×10^6
GST	not detected

[1] Used as positive control

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CLAIMS:

1. A nucleic acid molecule comprising a nucleotide sequence encoding, or complementary to a sequence encoding, an ovine cytokine-like molecule.

2. The nucleic acid molecule according to claim 1 wherein the ovine cytokine-like molecule is IFNγ or GM-CSF.

3. The nucleic acid molecule according to claim 1 wherein the ovine cytokine-like molecule is IL-1, IL-2 or IL-4.

4. The nucleic acid molecule according to claim 1 wherein the ovine cytokine-like molecule is TNFα or TNFβ.

5. The nucleic acid molecule according to claim 1 wherein the nucleotide sequence comprises deoxyribonucleotides.

6. The nucleic acid molecule according to claim 1 wherein the nucleotide sequence comprises ribonucleotides.

7. The nucleic acid molecule according to claim 5 wherein the molecule is a double stranded cDNA or synthetic DNA molecule.

8. The nucleic acid molecule according to any one of claims 1 to 7 wherein the molecule is contained in an expression vector which is capable of expressing the nucleotide sequence in a prokaryotic and/or eukaryotic cell.

9. A recombinant ovine cytokine-like molecule.

10. The recombinant ovine cytokine according to claim 9 wherein said cytokine is IFNγ or GM-CSF.

11. The recombinant ovine cytokine according to claim 9 wherein said cytokine is IL-1, IL-2 or IL-4.

12. The recombinant ovine cytokine according to claim 9 wherein said cytokine is TNFα or TNFβ.

13. An adjuvant composition comprising one or more recombinant ovine cytokine-like molecules mixed with or coupled to an antigen.

14. The composition according to claim 13 wherein at least one of the cytokines is GM-CSF and/or IFNγ.

15. The composition according to claim 13 wherein at least one of the cytokines is IL-1, IL-2 and/or IL-4.

16. The composition according to claim 13 wherein at least one of the cytokines is TNFα and/or TNFβ.

17. The composition according to any one of claims 13 to 16 further comprising one or more carriers and/or diluents acceptable for veterinary use.

18. A method for the treatment and/or prophylaxis of a livestock animal exposed to or infected with a pathogenic organism comprising administering to said animal an immunoresponsive effective amount of an ovine cytokine-like molecule for a time and under conditions sufficient to maintain, stimulate or enhance the immunoresponsiveness of said animal.

19. The method according to claim 18 wherein the cytokine-like molecule is a recombinant molecule.

20. The method according to claim 18 or 19 wherein the cytokine-like molecule is selected from one or more of IFNγ, GM-CSF, IL-1, IL-2, IL-4, TNFα and/or TNFβ.

21. The method according to claim 18 wherein the animal is a sheep.

22. A method of enhancing and/or stimulating an immune response to one or more antigens in an animal comprising administering to said animal an immunoresponsive effective amount of an ovine cytokine-like molecule.

23. The method according to claim 22 wherein the ovine cytokine-like molecule is a recombinant molecule.

24. The method according to claim 22 or 23 wherein the cytokine-like molecule is selected from one or more of IFNγ, GM-CSF, IL-1, IL-2, IL-4, TNFα and/or TNFβ.

25. The method according to claim 22 wherein the animal is a sheep.

26. The method according to any one of claims 22 to 25 further comprising the administration of one or more antigens.

27. An adjuvant nucleic acid molecule comprising a first nucleic acid encoding an ovine cytokine-like molecule inserted in a viral or bacterial expression vector together with a second nucleic acid molecule encoding an antigen or antigenic epitope such that both the cytokine and antigen are expressed.

28. The adjuvant nucleic acid molecule of claim 27 wherein the ovine cytokine-like molecule is selected from one or more of IFN-γ, GM-CSF, IL-1, IL-2, IL-4, TNFα and/or TNFβ.

29. A veterinary composition comprising one or more recombinant ovine cytokine-like molecules and one or more carrier and/or diluents acceptable for veterinary use.

30. The composition according to claim 29 wherein at least one of the cytokine-like molecules is GM-CSF and/or IFN-γ.

31. The composition according to claim 29 wherein at least one of the cytokine-like molecules is IL-1, IL-2 and/or IL-4.

32. The composition according to claim 29 wherein at least one of the cytokine-like molecules is TNFα and/or TNFβ.

33. The use of an ovine cytokine-like molecule in the manufacture of a medicament for the treatment and/or prophylaxis of an animal exposed to or infected with a pathogenic organism.

34. The use of an ovine cytokine-like molecule in the manufacture of a medicament to enhance and/or stimulate an immune response to one or more antigens in an animal.

35. The use according to claim 33 or 34 wherein the cytokine-like molecule is one or more of GM-CSF, IFN-γ, IL-1, IL-2, IL-4, TNFα or TNFβ.

36. The use according to claim 33 or 34 wherein the animal is a livestock animal.

37. The use according to claim 36 wherein the animal is a sheep.

38. A process for preparing a recombinant ovine cytokine-like molecule comprising transforming an appropriate cell with the nucleic acid molecule according to claim 8 and culturing the transformed cell for a time and under conditions suitable for synthesis of the ovine cytokine-like molecule.

39. The process according to claim 38 further comprising isolating the ovine cytokine-like molecule so produced.

SEQUENCE OF OVINE GAMMA-INTERFERON GENE

```
S1   Met Lys Tyr Thr Ser Tyr Phe Leu Ala Leu Leu Leu Cys Val Leu Leu   S16
 1   ATG AAA TAT ACA AGC TAT TTC TTA GCT TTA CTG CTC TGT GTG CTT TTG    48
     ----------------▼------------------ (IFN-N)
S17  Gly Phe Ser Gly Ser Tyr Gly Gln Gly Pro Phe Phe Lys Glu Ile Glu    12
49   GGT TTT TCT GGT TCT TAT GGC CAG GGC CCA TTT TTT AAA GAA ATA GAA    96
                ▲
13   Asn Leu Lys Glu Tyr Phe Asn Ala Ser Asn Pro Asp Val Ala Lys Gly    28
97   AAC TTA AAG GAG TAT TTT AAT GCA AGT AAC CCA GAT GTA GCT AAG GGT   144

29   Gly Pro Leu Phe Ser Glu Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp    44
145  GGG CCT CTC TTC TCA GAA ATT TTG AAG AAT TGG AAA GAG GAG AGT GAC   192

45   Lys Lys Ile Ile Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe    60
193  AAA AAG ATT ATT CAG AGC CAA ATT GTC TCC TTC TAC TTC AAA CTC TTT   240
```

Fig.1.

```
 61  Glu Asn Leu Lys Asp Asn Gln Val Ile Gln Arg Ser Met Asp Ile Ile    76
241  GAA AAC CTC AAA GAT AAC CAG GTC ATT CAA AGG AGC ATG GAT ATC ATC   288

 77  Lys Gln Asp Met Phe Gln Lys Phe Leu Asn Gly Ser Ser Glu Lys Leu    92
289  AAG CAA GAC ATG TTT CAG AAG TTC TTG AAC GGC AGC TCT GAG AAA CTG   336

 93  Glu Asp Phe Lys Arg Leu Ile Gln Ile Pro Val Asp Asp Leu Gln Ile   108
337  GAG GAC TTC AAA AGG CTG ATT CAA ATT CCG GTG GAT GAT CTG CAG ATC   384

109  Gln Arg Lys Ala Ile Asn Glu Leu Ile Lys Val Met Asn Asp Leu Ser   124
385  CAG CGC AAA GCC ATC AAT GAA CTC ATC AAG GTG ATG AAT GAC CTG TCG   432

125  Pro Lys Ser Asn Leu Arg Lys Arg Lys Arg Ser Gln Asn Leu Phe Arg   140
433  CCA AAA TCT AAC CTC AGA AAG CGG AAG AGA AGT CAG AAT CTC TTT CGA   480
                                          (IFN-C) ----------

141  Gly Arg Arg Ala [  Ser Thr End ]
481  GGC CGG AGA GC  [A TCA ACG     ]
     -------------        T
                        Met
```

Fig.1 cont.

COMPARISON OF GAMMA-INTERFERON AMINO ACID SEQUENCES

	S1								S10									S20	1										
Ovine	M	K	Y	T	S	Y	F	L	A	L	L	L	C	V	L	L	G	F	S	G	S	Y	G	Q	G	P	F	F
Bovine	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	Q	-	-
Human	-	-	-	-	-	-	I	-	-	F	Q	-	-	I	V	-	-	S	L	-	C	-	C	-	D	-	Y	V
Murine	-	N	A	-	H	C	I	-	-	-	Q	-	F	L	M	A	V	S	G	/	C	-	C	H	-	T	V	I

	10									20								30										
Ovine	K	E	I	E	N	L	K	E	Y	F	N	A	S	N	P	D	V	A	K	G	G	P	L	F	S	E	I	L
Bovine	R	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human	-	-	A	-	-	-	-	K	-	-	-	-	G	H	S	-	-	-	D	N	-	T	-	-	L	G	-	-
Murine	E	S	L	-	S	-	N	N	-	-	-	S	-	G	I	-	-	E	E	K	/	S	-	-	L	D	-	W

	40									50										60								
Ovine	K	N	W	K	E	E	S	D	K	K	I	I	Q	S	Q	I	V	S	F	Y	F	K	L	F	E	N	L	K
Bovine	-	-	-	-	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human	-	-	-	-	-	-	-	-	-	R	-	-	M	-	-	-	-	-	-	-	-	-	-	-	K	-	F	-
Murine	R	-	-	Q	K	D	G	-	M	-	-	L	-	-	-	-	I	-	-	-	L	R	-	-	-	V	-	-

Fig.2.

```
                70                  80                  90
Ovine    DNQVIQRSMDIIKQDMFQKFLNGSSEKL
Bovine   --------------------------
Human    --D--S---K-VET---E---NV---F-SNKK-R
Murine   ---A-SNNISV-ESHLITT-FSN-KA-K

               100                 110                 120
Ovine    EDFKRLIQIPVDDLQIQRKAINELIKVM
Bovine   --------------------------
Human    D---TNYS--T---NV----------Q---
Murine   DA-MSIAKFE-NNP-V---Q-F-----R-V

               130                 140                 146
Ovine    NDLSPKSNLRKRKRSQNLFR/GRRAS(M/T)
Bovine   --------------------------
Human    AE---AAKTG-------M-----/-----Q
Murine   HQ-L-E-S-------RCW-GV-K-L-Q
```

Fig.2 cont.

SEQUENCE OF OVINE GM-CSF GENE

```
1       ATG TGG CTG CAG AAC CTG CTT CTC CTG GGC ACT GTG GTC TGC    42
S1      Met Trp Leu Gln Asn Leu Leu Leu Leu Gly Thr Val Val Cys    S14
        ---------------------------------- GM-N

43      AGC TTC TCC GCA CCC ACT CGC CAA CCC AGC CCT GTC ACC CGG    84
S15     Ser Phe Ser Ala Pro Thr Arg Gln Pro Ser Pro Val Thr Arg    11

85      CCC TGG CAG CAT GTG GAT GCC ATC AAG GAG GCC CTG AGC CTT    126
12      Pro Trp Gln His Val Asp Ala Ile Lys Glu Ala Leu Ser Leu    25

127     CTG AAC GAC AGC ACT GAC ACT GCT GCT GTG ATG GAT GAA ACA    168
26      Leu Asn Asp Ser Thr Asp Thr Ala Ala Val Met Asp Glu Thr    39

169     GTA GAA GTC GTC TCT GAA ATG TTT GAC TCC CAG GAG CCG ACA    210
40      Val Glu Val Val Ser Glu Met Phe Asp Ser Gln Glu Pro Thr    53

211     TGC CTG CAG ACT CGC CTG GAG CTG TAC AAG CAG GGC CTG CGG    252
54      Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys Gln Gly Leu Arg    67
```

Fig.3.

```
253   GGC AGC CTC ACC AGT CTC ACG GGC TCC TTG ACC ATG ATG GCC   294
68    Gly Ser Leu Thr Ser Leu Thr Gly Ser Leu Thr Met Met Ala    81

295   AGC CAC TAC AAG AAA CAC TGC CCC CCC ACC CAG GAA ACT TCC   336
82    Ser His Tyr Lys Lys His Cys Pro Pro Thr Gln Glu Thr Ser    95

337   TGT GAA ACC CAG ATT ATC ACC TTC AAA AGT TTC AAA GAG AAC   378
96    Cys Glu Thr Gln Ile Ile Thr Phe Lys Ser Phe Lys Glu Asn   109

379   CTG AAG GAT TTC CTT TTT ATC ATT CCC TTT GAC TGC TGG GAA   420
110   Leu Lys Asp Phe Leu Phe Ile Ile Pro Phe Asp Cys Trp Glu   123
                                        GM-C ----------------

421   CCA GCC CAG AAG        435
124   Pro Ala Gln Lys STOP   129
      --------------
```

Fig. 3 cont.

COMPARISON OF GM-CSF AMINO ACID SEQUENCES

	S1								S10								1								10
Ovine	M	W	L	Q	N	L	L	L	L	G	T	V	V	C	S	F	S	A	P	T	R	Q	P	S	P	V	T
Bovine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	N	T	A	-
Human	-	-	-	-	S	-	-	-	-	-	-	A	-	-	I	-	-	-	A	-	S	-	-	-	S	-	
Murine	-	-	-	-	-	-	-	-	F	-	-	I	-	-	Y	-	L	-	-	-	-	-	S	-	I	T	-	-

									20								30								
Ovine	R	P	W	Q	H	V	D	A	I	K	E	A	L	S	L	L	N	D	S	T	D	T	A	A	V	M	D
Bovine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	-	S	-	-	D	-	-	-	N
Human	Q	-	-	E	-	-	N	-	I	Q	-	-	R	R	-	-	-	L	-	R	-	-	-	-	E	-	N
Murine	-	-	-	K	-	-	E	-	-	-	-	-	-	N	-	-	D	/	/	/	-	M	P	V	T	L	N

			40								50								60							
Ovine	E	T	V	E	V	V	S	E	M	F	D	S	Q	E	P	T	C	L	Q	T	R	L	E	L	Y	K	Q
Bovine	D	-	/	-	-	-	-	-	K	-	-	-	-	-	-	-	-	-	-	-	-	-	K	-	-	-	N
Human	-	-	-	-	-	-	I	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Murine	-	E	-	-	-	-	-	-	N	E	-	S	F	K	K	L	-	-	V	-	-	-	-	K	I	F	E	-

Fig.4.

```
                70                  80                  90
Ovine     GLRGSLTSLTGSLTMMASHYKKHCPPT
Bovine    --Q-------M-------T---E-----
Human     ------K-K-P-------Q---------
Murine    ----NF-K-K-A-N-T---Y-QTY----

               100                 110
Ovine     QETSCETQIITFKSFKENLKDFLFIIP
Bovine    P---G--F--S---N-----D---E---
Human     P-----A-------E-------------
Murine    P---D-----VT-YAD-IDS---T---

               120
Ovine     FDCWEPAQK
Bovine    ---------
Human     -------V-E
Murine    -E-KK--S--
```

Fig.4cont.

SEQUENCE OF OVINE IL-2 GENE

1	Met Tyr Lys Ile Gln Leu Leu Ser Cys Ile Ala Leu Thr Leu Ala Leu	16
1	ATG TAC AAG ATA CAA CTC TTG TCT TGC ATT GCA CTA ACT CTT GCA CTC	48
17	Val Ala Asn Gly Ala Pro Thr Ser Ser Ser Thr Gly Asn Thr Met Lys	32
49	GTT GCA AAC GGT GCA CCT ACT TCA AGC TCT ACG GGG AAC ACA ATG AAA	96
33	Glu Val Lys Ser Leu Leu Leu Asp Leu Gln Leu Leu Leu Glu Lys Val	48
97	GAA GTG AAG TCA TTG CTG CTG GAT TTA CAG TTG CTT TTG GAG AAA GTT	144
49	Lys Asn Pro Glu Asn Leu Lys Leu Ser Arg Met His Thr Phe Asn Phe	64
145	AAA AAT CCC GAG AAC CTC AAG CTC TCC AGG ATG CAT ACA TTT AAC TTC	192
65	Tyr Met Pro Lys Val Asn Ala Thr Glu Leu Lys His Leu Lys Cys Leu	80
193	TAC ATG CCC AAG GTT AAC GCT ACA GAA TTG AAA CAT CTT AAG TGT TTA	240

Fig.5

81	Leu Glu Glu Leu Lys Leu Leu Glu Glu Val Leu Asp Leu Ala Pro Ser	96
241	CTA GAA GAA CTC AAA CTT CTA GAG GAA GTG CTA GAT TTA GCT CCA AGC	288
97	Lys Asn Leu Asn Thr Arg Glu Ile Lys Asp Ser Met Asp Asn Ile Lys	112
289	AAA AAC CTG AAC ACC AGA GAG ATC AAG GAT TCA ATG GAC AAT ATC AAG	336
113	Arg Ile Val Leu Glu Leu Gln Gly Ser Glu Thr Arg Phe Thr Cys Glu	128
337	AGA ATA GTT TTG GAA CTA CAG GGA TCT GAA ACA AGA TTC ACA TGT GAA	384
129	Tyr Asp Asp Ala Thr Val Lys Ala Val Glu Phe Leu Asn Lys Trp Ile	144
385	TAT GAT GAT GCG ACA GTA AAG GCT GTA GAA TTT CTG AAC AAA TGG ATT	432
145	Thr Phe Cys Gln Ser Ile Tyr Ser Thr Met Thr ...	
433	ACC TTT TGT CAA AGC ATC TAC TCA ACA ATG ACT TGA	

Fig.5cont.

COMPARISON OF IL-2 AMINO ACID SEQUENCES

```
OVINE     MYKIQLLSCI ALTLALVANG APTSSSTGNT MKE.......  .......VKS   36
BOVINE    ---------- ---------- ---------- ---.......  .......---
HUMAN     --RM------ --S----T-S -------KK- QLQ.......  .......LEH
MURINE    --SM--A--V T---V-LV-S -------SSS TA-AQQQQQQ  QQQQQQHLEQ

OVINE     LLLDLQLLLE KVKNPENLKL SRMHTFNFYM PKVNATELKH  LkCLLEELKL   86
BOVINE    ---------- ---------- ------D--V ----------  ----------
HUMAN     ------MI-N GIN-YK-P-- T--L--K---  --K.------  -Q--E----P
MURINE    --M---E--S RME-YR---- P--L--K--L  --Q.-----D  -Q--ED--GP

OVINE     LEEVLDLAPS KNLNTREIKD SMDNTKRIVL ELQGSETRFT  CEYDDATVKA  136
BOVINE    -----N---- ----P----- ---------- ----------  --------N-
HUMAN     -----N--Q- --FHL.RPR- LIS--NV--- --K----T-M  ---A-E-ATI
MURINE    -RH----TQ-  SFQLEDAEN FIS--RVT-V K-K--DNT-E  -QF--ESATV

OVINE     VEFLNKWITF CQSIYSTMT.                         155
BOVINE    ---------- ---------.
HUMAN     -----R---- ----I--L-.
MURINE    -D--RR--A- ----I--SPQ
```

Fig.6.

NUCLEOTIDE SEQUENCE OF OVINE IL-1β cDNA

```
  1  M   A   T   V   P   E   P   I   N   E   V   M   A   Y   Y
  1 ATG GCA ACC GTA CCT GAA CCC ATT AAT GAA GTG ATG GCT TAC TAC

 16  Y   S   D   E   N   E   L   L   F   E   V   D   G   P   K
 48 TAC AGT GAT GAG AAT GAG CTG TTA TTT GAG GTT GAT GGC CCC AAA

 31  Q   M   K   S   C   T   Q   H   L   D   L   G   S   M   G
 93 CAG ATG AAG AGC TGC ACC CAA CAC CTG GAC CTC GGC TCC ATG GGA

 46  D   G   N   I   Q   L   Q   I   S   H   Q   L   Y   N   K
138 GAT GGA AAC ATC CAG CTG CAG ATT TCT CAC CAG CTC TAC AAC AAA

 61  S   F   R   Q   V   V   S   V   I   V   A   M   E   K   L
183 AGC TTC AGG CAG GTA GTG TCG GTC ATC GTG GCC ATG GAG AAG CTG

 76  R   S   R   A   Y   E   H   V   F   R   D   D   D   L   R
228 AGG AGC CGT GCC TAC GAA CAT GTC TTC CGT GAT GAT GAC CTG AGG

 91  S   I   L   S   F   I   F   E   E   E   P   V   I   F   E
273 AGC ATC CTT TCA TTC ATC TTC GAA GAA GAG CCT GTC ATC TTC GAA

106  T   S   S   D   E   L   L   C   D   A   A   V   Q   S   V
318 ACA TCC TCC GAT GAG CTT CTG TGT GAT GCA GCC GTG CAG TCA GTA

121  K   C   K   L   Q   D   R   E   Q   K   S   L   V   L   D
363 AAA TGC AAA CTC CAG GAC AGA GAG CAA AAA TCC CTG GTG CTG GAT
```

Fig.7

```
136  S   P   C   V   L   K   A   L   H   L   P   S   Q   E   M
408 AGC CCA TGT GTG CTG AAG GCT CTC CAC CTC CCC TCA CAG GAA ATG

151  S   R   E   V   V   F   C   M   S   F   V   Q   G   E   E
453 AGC CGA GAA GTG GTG TTC TGC ATG AGC TTC GTA CAA GGA GAG GAA

165  R   D   N   K   I   P   V   A   L   G   I   R   D   K   N
495 AGA GAC AAC AAG ATT CCT GTG GCC TTG GGT ATC AGG GAC AAG AAT

180  L   Y   L   S   C   V   K   K   G   D   T   P   T   L   Q
540 CTA TAC CTG TCT TGT GTG AAA AAA GGT GAT ACA CCG ACC CTG CAG

195  L   E   E   V   D   P   K   V   Y   P   K   R   N   M   E
585 CTG GAG GAA GTA GAC CCC AAA GTC TAC CCC AAG AGG AAT ATG GAA

210  K   R   F   V   F   Y   K   T   E   I   K   N   T   V   E
630 AAG CGA TTC GTC TTC TAC AAG ACA GAA ATC AAG AAC ACA GTT GAA

225  F   E   S   V   L   Y   P   N   W   Y   I   S   T   S   Q
675 TTT GAG TCT GTC CTG TAC CCT AAC TGG TAC ATC AGC ACT TCT CAA

240  I   E   E   K   P   V   F   L   G   R   F   R   G   G   Q
720 ATC GAA GAA AAG CCC GTC TTC CTG GGA CGT TTT AGA GGT GGC CAG

255  D   I   T   D   F   R   M   E   T   L   S   P  ...
765 GAT ATA ACT GAC TTC AGA ATG GAA ACC CTC TCT CCC TAG
```

Fig 7.cont.

COMPARISON OF OVINE BOVINE HUMAN AND MURINE IL-1β AMINO ACID SEQUENCES

```
                1                  10                  20
OVINE     M A T V P E P I N E V M A Y Y S D E N E . L L F E V D
BOVINE    - - - - - - - - M - - - - - - - - - - - - - - - A -
HUMAN     - - E - - K L A S - M - - - G N E D D - F - - A - -
MURINE    - - - - - L N C - M P P F D - - - - D . - F - - - -

                30                  40                  50
OVINE     G P K Q M K S C T Q H L D L G S M G D G N I Q L Q I S
BOVINE    D - - - - - - I - - - - - - - - - - - - - - - - - -
HUMAN     - - - - - C S F - D - - - C P L . - - G - - - R - -
MURINE    - - Q K - - G - F - - T F - - - C P . - E S - - - - -

                60                  70
OVINE     H Q L Y N K S F R Q V V S V I V A M E K L R . S R A Y
BOVINE    - - F - - - - - - - - - - - - - - - - - - N - . - - -
HUMAN     D H H - S - G - - - A A - - V - - - D - - - K M L V P
MURINE    Q - H I - - - - - - A - - L - - - V - - - W Q L P V S
```

Fig.8.

```
                80                  90                  100
OVINE     E . HVFRDDDERSILSFIFEEEPVIFET
BOVINE    . - -H- - - - - - - - - - - - -
HUMAN     . CPQT-QEN- -STFFFP- - - - -IF-D-
MURINE    FPWT-Q-E-MSTFFF- - - - - - -ILCDS

                110                 120                 130
OVINE     . SSDELLCDAAVQSVKCKLQDREQKSL
BOVINE    . - -F- - -P- - -I- - - - - - - -
HUMAN     . WDNEAYVH- -P- -R-LN-T-R-SQ- - -
MURINE    WDD-DNV- -VPIRQLHYR-R-EQ- - -

                140                 150
OVINE     VLDSPCVLKALHLPSQEMSREVVFCMS
BOVINE    - -A- - - - - - - - - -L- - -N- - - - - -
HUMAN     -MSG-YE- - - - - -QG-D-EQQ- - -S- - -
MURINE    - -SD-YE- - - - - -NG-N-INQQ-I- -S- -

                160                 170                 180
OVINE     FVQGEERDNKIPVALGIRDKNLYLSCV
BOVINE    - - - - - - - - - - - - - - - - - - - - - - - - - - -
HUMAN     - - - - - - -SND- - - - - - -LKE- - - - - - - - - -
MURINE    - - - - - - -PSND- - - - - - -LKG- - - - - - - - - -
```

Fig.8 cont.

```
                190                 200                 210
OVINE    K K G D T P T L Q L E E V D P K V Y P K R N M E K R F
BOVINE   - - - - - - - - - - - - - - - - - - - - - - - - - -
HUMAN    L - D - K - - - - - - S - - - - - - N - - - - K K - -
MURINE   M - D G - - - - - - - S - - - - - - Q - - - - K K - -

                220                 230
OVINE    V F Y K T E I K N T V E F E S V L Y P N W Y I S T S Q
BOVINE   - - - - - - - - - - - - - - - - - - - - - - - - - -
HUMAN    - - - N - - I - - N - K L - - - - - - A Q F - - - - -
MURINE   - - - N - - I - - V - - S K - - - - - A E F - - - - -

                240                 250                 260
OVINE    I E E K P V F L G R F R G G Q D I T D F R M E T L S P
BOVINE   - - - - R - - - - - - H - - - - - - - - - - - - - -
HUMAN    A - - N M - - - - - - G T K - - - - - - - T - - Q F V - - S
MURINE   A - - H K - - - - - - . N N S - - - - - I - - T - - S V - - S
```

Fig.8cont.

NUCLEOTIDE AND INFERRED AMINO ACID SEQUENCE OF OVINE TNF-α cDNA

```
  1  Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Val   16
  1  ATG AGC ACA GAA AGC ATG ATC CGG GAT GTG GAG CTG GCG GAG GAG GTG   48

 17  Leu Ser Asn Lys Ala Gly Gly Pro Gln Gly Ser Arg Ser Cys Trp Cys   32
 49  CTC TCC AAC AAA GCA GGG GGC CCC CAG GGC TCC AGA AGT TGC TGG TGC   96

 33  Leu Ser Leu Phe Ser Phe Leu Leu Val Ala Gly Ala Thr Thr Leu Phe   48
 97  CTC AGC CTC TTC TCC TTC CTC CTG GTT GCA GGA GCC ACC ACG CTC TTC  144

 49  Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Ser Pro   64
145  TGC CTG CTG CAC TTC GGG GTA ATC GGC CCC CAG AGG GAA GAG TCC CCA  192

 65  Ala Gly Pro Ser Phe Asn Arg Pro Leu Val Gln Thr Leu Arg Ser Ser   80
193  GCT GGC CCC TCC TTC AAC AGG CCT CTG GTT CAG ACA CTC AGG TCA TCT  240

 81  Ser Gln Ala Ser Asn Asn Lys Pro Val Ala His Val Val Ala Asn Ile   96
241  TCT CAA GCC TCA AAT AAC AAG CCG GTA GCC CAC GTT GTA GCC AAC ATC  288

 97  Ser Ala Pro Gly Gln Leu Arg Trp Gly Asp Ser Tyr Ala Asn Ala Leu  112
289  AGC GCT CCG GGG CAG CTC CGA TGG GGG GAC TCG TAT GCC AAT GCC CTC  336
```

Fig.9

```
113  Met Ala Asn Gly Val Glu Leu Lys Asp Asn Gln Leu Val Val Pro Thr  128
337  ATG GCC AAC GGC GTG GAG CTG AAA GAC AAC CAG CTG GTG GTG CCC ACT  384

129  Asp Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Arg Gly His Gly  144
385  GAC GGG CTT TAC CTC ATC TAC TCA CAG GTC CTC TTC AGG GGC CAC GGC  432

145  Cys Pro Ser Thr Pro Leu Phe Leu Thr His Thr Ile Ser Arg Ile Ala  160
433  TGC CCT TCC ACC CCC TTG TTC CTC ACC CAC ACC ATC AGC CGC ATT GCA  480

161  Val Ser Tyr Gln Thr Lys Val Asn Ile Leu Ser Ala Ile Lys Ser Pro  176
481  GTC TCC TAC CAG ACC AAG GTC AAC ATC CTC TCT GCC ATC AAA AGC CCT  528

177  Cys His Arg Glu Thr Leu Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu  192
529  TGC CAC AGG GAG ACC CTA GAG GGG GCT GAG GCC AAG CCC TGG TAC GAA  576

193  Pro Ile Tyr Gln Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu  208
577  CCC ATC TAC CAG GGA GGG GTC TTC CAG CTG GAG AAG GGA GAT CGC CTC  624

209  Ser Ala Glu Ile Asn Leu Pro Glu Tyr Leu Asp Tyr Ala Glu Ser Gly  224
625  AGT GCT GAG ATC AAC CTG CCG GAA TAC CTG GAC TAT GCC GAG TCT GGG  672

225  Gln Val Tyr Phe Gly Ile Ile Ala Leu ...
673  CAG GTC TAC TTT GGG ATC ATC GCC CTG TGA
```

Fig. 9 cont.

COMPARISON OF TNF-α AMINO ACID SEQUENCES

```
                1                   10                  20
Ovine     M S T E S M I R D V E L A E E V L S N K A G G P Q G S R
Bovine    - - K - - - - - - - - - - - - - - S E - - - - - - - - -
Human     - - - - - - - - - - - - - - - - A - P K - T - - - - - -
Mouse     - - - - - - - - - - - - - - - - A - P Q - M - - F - N - -
Rabbit    - - - - - - - - - - - - - - - - P - P K - - - - - - - K

                30                  40                  50
Ovine     S C W C L S L F S F L L V A G A T T L F C L L H F G V I
Bovine    - - L - - - - - - - - - - - - - - - - - - - - - - - - -
Human     R - L F - - - - - - - - - I - - - - - - - - - - - - - -
Mouse     R - L - - - - - - - - - - - - - - - - - - - - N - - - -
Rabbit    R - L - - - - - - - - - - - - - - - - - - - - - R - - -

                60                  70                  80
Ovine     G P Q R E E • S P A G P S F N R P L V Q T • • L R S S S
Bovine    - - - - - - Q - - - - - - I - - S - - - - • - - - - - -
Human     - - - - - - F • - R D L - L I S - - A - A • • V - - - -
Mouse     - - - - - D - K F - N - L P L I S S M A - - L T - - - -
Rabbit    - - - E - - Q - - N N L H L V N - V A - M V T - - - A -
```

Fig.10.

```
                                90                  100
Ovine    QASNNKPVAHVVANISAPGQLRWGDSYA
Bovine   -S-----D-NS-----W---
Human    RTPSSD-----PQAE----Q-LNRR-
Mouse    QN-SSO-----HQVEE---E-LSQR-
Rabbit   R-LSSD-----PQVE----Q-LSQR-

                    110                 120                 130
Ovine    NALMANGVELKDNQLVVPTDGLYLIYSQ
Bovine   ---------K-E-----------A-E---
Human    ---------R-----------S-E---
Mouse    ---L---L-M-D-----------A---V---
Rabbit   ---L---L-M-K-T---------A---

                    140                 150                 160
Ovine    VLFRGHGCPSTPLFLTHTISRIAVSYQT
Bovine   ---Q-----P-PV----------------
Human    ---K-Q-----H-VL--------------
Mouse    ---K-Q-----D-YVL-------V---F---I---E
Rabbit   ---S-Q-----R-SYVL-------V---F---P-N
```

Fig.10 cont.

```
                170                 180                 190
Ovine   K V N I L S A I K S P C H R E T L E G A E A K P W Y E P
Bovine  - - - - - - - - - - - - - - P - W - - - - - - - - - - -
Human   - - - L - - - - - - - - - - P - - - - - - - - - - - - -
Mouse   - - - L - - - V - - - - - - P K D - P - G - - - L - - - -
Rabbit  - - - L - - - - - - - - - - P - E - - P M A - - - - - -

                200                 210                 220
Ovine   I Y Q G G V F Q L E K G D R L S A E I N L P E Y L D Y A
Bovine  - - - - - - - - - - - - - - - - - - - - - - D - - - - -
Human   - - - L - - - - - - - - - - - - - - - - - R - D - - - F -
Mouse   - - - L - - - - - - - - - - Q - - - - - V - - K - - - F -
Rabbit  - - - L - - - - - - - - - - - - - - - T - V - - Q - - - L -

                230
Ovine   E S G Q V Y F G I I A L
Bovine  - - - - - - - - - - - -
Human   - - - - - - - - - - - -
Mouse   - - - - - - - - - - - -
Rabbit  - - - - - - - - - - - -
```

Fig.10 cont.

NUCLEOTIDE AND INFERRED AMINO ACID SEQUENCE OF OVINE TNF-β cDNA SEQUENCE

```
                                   (G)
 1   M  T  P  P  E  R  L  C  L  L  R  V  C  S  T  P  S  L  L  L     20
 1   ATGACACCACCTGAACGTCTCTGCCTCCTGAGGGTGTGCAGCACCCCATCCCTCCTCCTC     60
                                   G

21   L  L  G  L  L  L  L  A  L  P  P  E  A  Q  G  L  R  G  V  G  L     40
61   CTCCTGGGGCTGCTGCTGGCCCTGCCGCCGGAGGCCCAGGGGCTCCGTGGCGTTGGCCTC    120

                                   (H)
41   T  P  S  A  A  E  P  A  H  Q  Q  L  P  K  P  F  T  R  G  T     60
121  ACACCCTCAGCTGCAGAGCCTGCCCATCAGCAGCTCCCGAAGCCCTTCACCCGTGGCACC    180
                                   A

61   L  K  P  A  A  H  L  V  G  D  P  S  T  Q  D  S  L  R  W  R     80
181  CTCAAACCCGCCGCTCACCTTGTTGGAGACCCCAGCACCCAGGACTCGCTGCGCTGGAGG    240

81   A  N  T  D  R  A  F  L  R  H  G  F  S  L  S  N  N  S  L  L    100
241  GCAAACACGGACCGCGCCTTCCTGCGCCATGGCTTCTCTCTCAGCAACAACTCCCTCCTG    300
```

Fig.11.

```
101   V  P  T  S  G  L  Y  F  D  Y  S  Q  V  V  F  S  G  K  G  C      120
301   GTCCCCACCAGTGGCCTGTACTTCGACTACTCCCAAGTGGTCTTCTCTGGGAAAGGCTGC      360

                                                      (P)
121   F  P  R  A  T  P  T  P  L  Y  L  A  H  E  V  Q  L  F  S  P      140
361   TTCCCCAGGGCCACCCCCACTCCTCTCTACCTGGCCCATGAGGTCCAGCTCTTCTCCCCA      420
                                                      C

141   Q  Y  P  F  H  V  P  L  L  S  A  Q  K  S  V  C  P  G  P  Q      160
421   CAGTATCCCTTCCATGTGCCTCTCCTCAGCGCTCAGAAGTCCGTGTGCCCAGGGCCACAG      480

161   G  P  W  V  Q  S  V  Y  Q  G  A  V  F  L  L  T  R  G  D  Q      180
481   GGGCCATGGGTGCAGTCGGTGTACCAGGGGGCTGTATTCCTGCTCACCAGGGGAGACCAG      540

181   L  S  T  H  T  D  G  I  S  H  L  L  L  S  P  S  T  V  F  F      200
541   CTATCCACTCACACAGACGGCATCTCCCACCTGCTCCTCAGCCCCAGTACTGTCTTCTTT      600

201   G  A  F  A  L  ·
601   GGAGCCTTCGCTCTGTAG
```

Fig.11 cont.

ALIGNMENT OF OVINE HUMAN AND MURINE TNF-β AMINO ACID SEQUENCES

```
                  1                  10                  20
                                    (G)
Ovine     M T P P E R L C L L R V C S T P S L L L L G L L L
Human     - - - - - - - F - P - V - G T T L H - - - - - - - -
Murine    - - L L G - - H - - G - - - - - P V F - - - - - - -

                        30          .       40                  50
                                                               (H)
Ovine     A L P P E A Q G L R G V G L T P S A A E P A H Q Q L
Human     V - L - G - - - - P - - - - - - - - - Q T - R - H P
Murine    - - - L G - - - - S - - - F . . - - - R T - - P L P

                                 60                  70
Ovine     P K P F T R G T L K P A A H L V G D P S T Q D S L R
Human     K M H L A H S - - - - - - - - I - - - - K - N N - L
Murine    Q - H L - H - I - - - - - - - V - Y - - K - N - - L

                  80                  90          +       100
Ovine     W R A N T D R A F L R H G F S L S N N S L L V P T S
Human     - - - - - - - - - - - Q D - - - - - - - - - - - - -
Murine    - - - S - - - - - - - - - - - - - - - - - - I - - -
```

Fig.12.

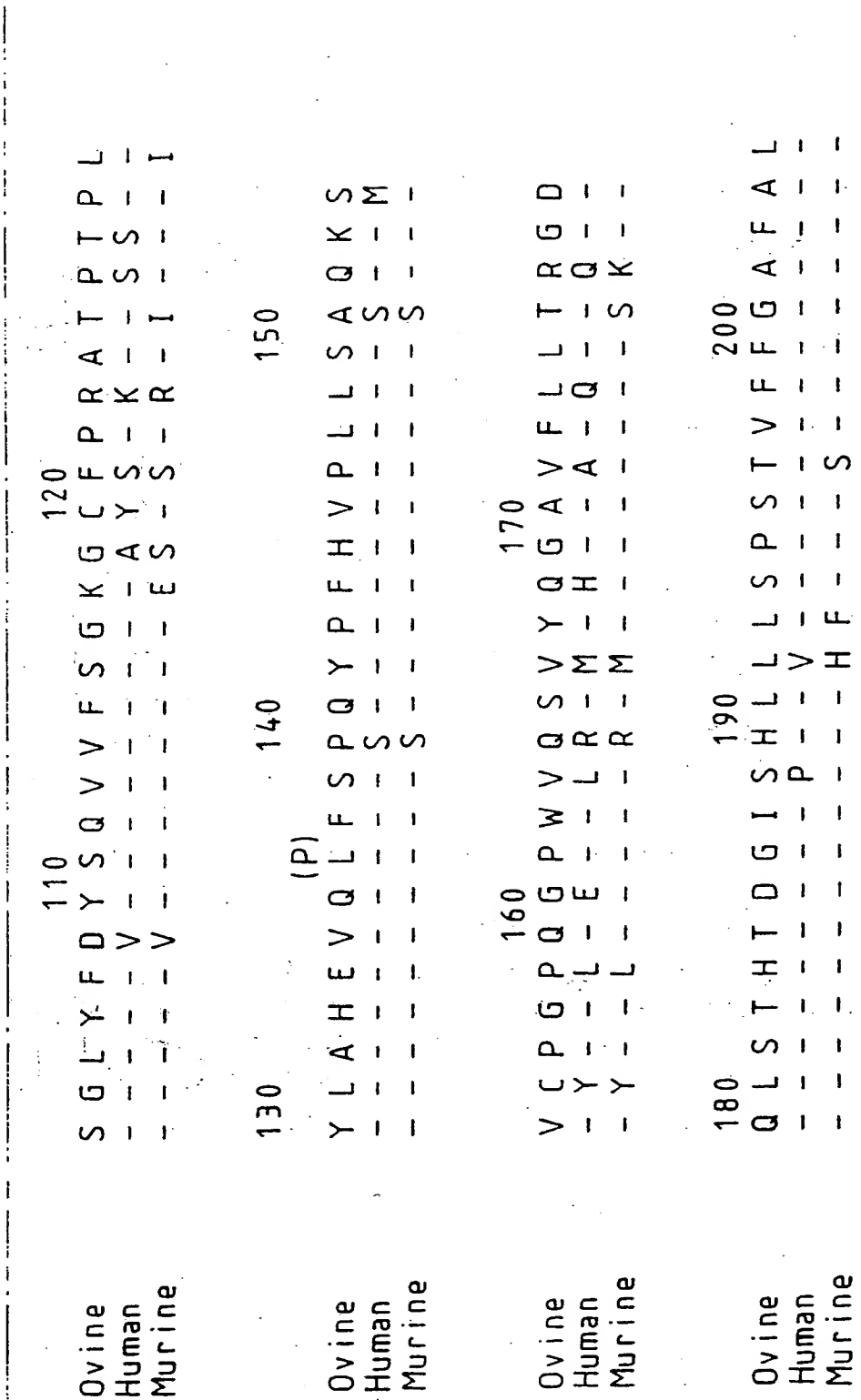
110
120
130
140
150
160
170
180
190
200
(P)
Ovine S G L Y F D Y S Q V V F S G K G C F P R A T P T P L
Human - - - - V - - - - - - - - - - - A Y S - K - - S S - -
Murine - - - - V - - - - - - - - - - E S - S - R - I - - - I
Ovine Y L A H E V Q L F S P Q Y P F H V P L L S A Q K S
Human - - - - - - - - - S - - - - - - - - - - S - - Q - -
Murine - - - - - - - - - S - - - - - - - - - - S - - S - -
Ovine V C P G P Q G P W V Q S V Y Q G A V F L L T R G D
Human - Y - - - L - - E - - L R - M - - H - - A - Q - - Q - -
Murine - Y - - - L - - - - - - R - - M - - - - - - - - S K - -
Ovine Q L S T H T D G I S H L L L S P S T V F F G A F A L
Human - - - - - - - - - - - - P - - - V - - - - - - - - - - -
Murine - - - - - - - - - - - - - - - H F - - - S - - - - - - -

Fig 12 cont.

NUCLEOTIDE AND INFERRED AMINO ACID SEQUENCE OF OVINE IL-4 cDNA

```
      TAGCTTCTCCTGATAATCTAATTGCCTCACACTGTCAGTGCAAATAGAGATACTATTA    58

 1    M  G  L  T  S  Q  L  I  P  A  L  V  C  L  L  V  C  T  S  H    20
59    ATGGGTCTCACCTCCCAGCTGATCCCAGCGCTGGTCTGCTTACTGGTATGTACCAGCCAC  118

21    F  V  H  G  H  K  C  D  I  T  L  E  E  I  I  K  T  P  N  I    40
119   TTCGTCCATGGACACAAGTGTGATATTACCTTAGAAGAGATCATCAAAACGCCGAACATC  178

41    L  T  S  R  K  N  S  C  M  E  L  P  V  A  D  V  F  A  A  P    60
179   CTCACATCGAGAAAGAATTCATGCATGGAGCTGCCTGTAGCAGACGTCTTTGCTGCCCCA  238

61    K  N  A  T  E  K  E  T  F  C  R  A  G  I  E  L  R  R  I  Y    80
239   AAGAACGCAACTGAGAAGGAAACCTTCTGCAGGGCTGGAATTGAGCTTAGGCGTATCTAC  298

81    R  S  H  M  C  L  N  K  F  L  G  G  L  D  R  N  L  S  S  L   100
299   AGGAGCCACATGTGCTTGAACAAATTCCTGGGCGGACTTGACAGGAATCTCAGCAGCCTG  358

101   A  S  K  T  C  S  V  N  E  A  K  T  S  T  S  T  L  R  D  L   120
359   GCAAGCAAGACCTGTTCTGTGAATGAAGCCAAGACGAGTACAAGTACGCTGAGAGACCTC  418

121   L  E  R  L  K  T  I  M  R  E  K  Y  S  K  C  ·
419   TTGGAAAGGCTAAAGACTATTATGAGGGAGAAATACTCAAAGTGTTGAAGCTGAATATTT  478

479   TAATTTATGACTTTTTAATAGCCTTATTTTAAACATTTTATATATTTATAACTCAT
```

Fig.13.

ALIGNMENT OF OVINE,
HUMAN, MURINE, AND RAT IL-4 AMINO ACID SEQUENCES

```
                                10                  20
Ovine     M G L T S Q L I P A L V C L L V C T S H F V H G
Human     - - - T S - - L - P - F F - - A - A G N - - - -
Murine    - - - N P - - V V I - I F F - E - - R S H I - -
Rat       - - - S P H - A V T - F - F - I - - G N G I - -

                          30                  40
Ovine     H K C D . I T L E E I I K T P N I L T S R K N S
Human     - - - - . - - - Q - - - - - - L - S - - E Q - T L
Murine    . . - - K N H - R - - - G I L - E V - G E G T P
Rat       . . - N D S P - R - - - N - L - Q V - E K G T P

                50                  60  +               70
Ovine     C M E L P V A D V F A A P K N A T E K E T F C R
Human     - T - - T - T - I - - - S - - T - - - - - - - -
Murine    - T - M D - P N - L T - T - - T - - S - L V - -
Rat       - T - M F - P - - L T - T R - T - - N - L I - -
```

Fig.14.

```
                         80                       90
Ovine    - G I E L R R I Y R S H . . . . M C L N K F L G
Human    - A T V - - Q F - S H - E K D T R - - G A T A Q
Murine   - S K V - - I F - L K - G K . T P - - K K N S S
Rat      - S R V - - K F - F P R D V . P P - - K N K S -

                                   100
Ovine    G L D R N . . L . . . S S L A S K . . . T C S .
Human    Q F H - H K Q - I R F - K R - D R N L W G L A G
Murine   V L M E L Q R - F - A F R C L - S . . . . . . .
Rat      V L G E L R K - C - G V - G L N S . . . . . . .

            110                   120
Ovine    V N E A K T S T S T L R D L . . . L E R L K T I
Human    L - - C P V K E A N Q S T - E N F - . - - - - -
Murine   S I - C T M N E - K S T S - K D F - - S - - S I
Rat      L R - C T V N E - - - T T - K D F - - S - - S I

           130
Ovine    M R E K Y S K C
Human    - - - - - - - - S S
Murine   - Q M D - -
Rat      L - G - - L Q S - T - M S
```

Fig.14 cont.

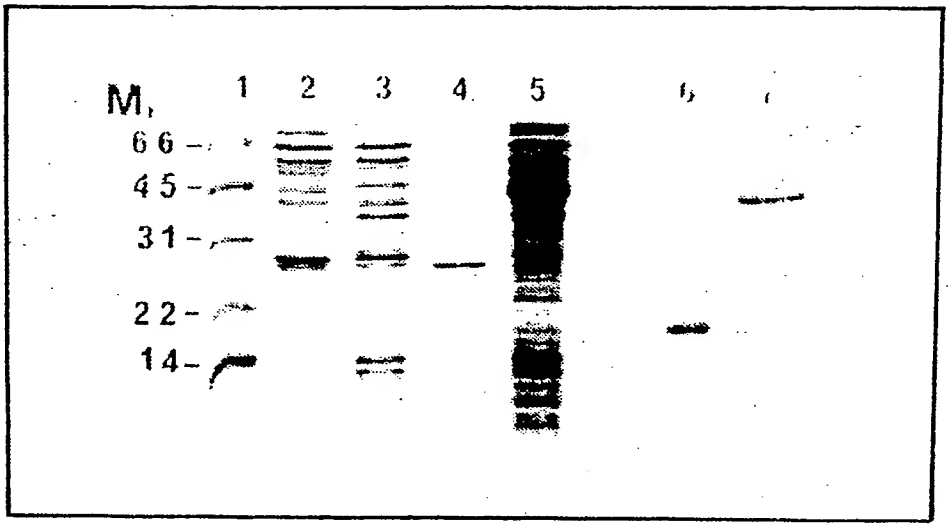

Fig. 15.

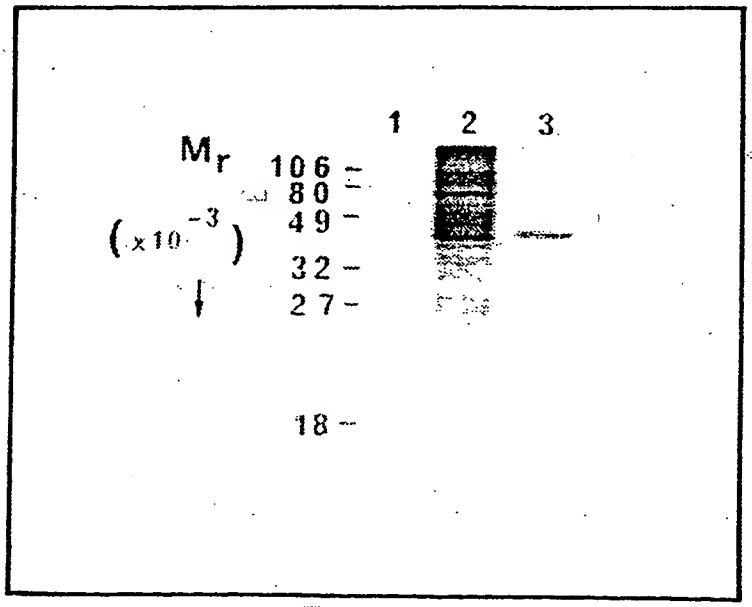

Fig. 16.

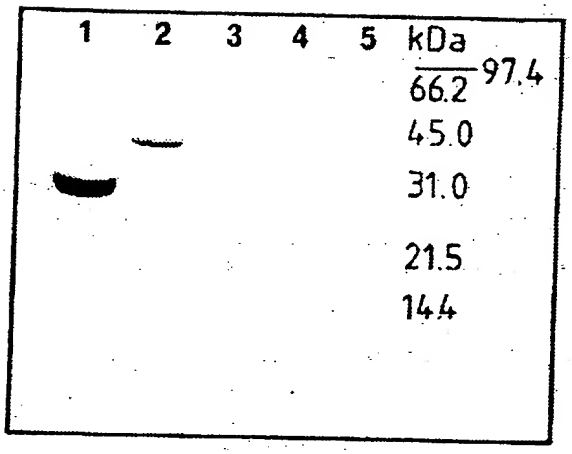

Fig.17.

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent classification (IPC) or to both National Classification and IPC
Int. Cl.[6] C12N 15/19, 15/20, 15/23, 15/24, 15/25, 15/26, 15/27, 15/28; A61K 37/02, 37/66, 39/39

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	WPAT, CAS 82: Keywords: Bovine, cow, ovine, sheep, livestock, rumin,
Chem. Abstr.	cytokin, interleukin, interferon, tumor necrosis factor, colony stimulating factor

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU : C12N 15/19, 15/20, 15/23, 15/24, 15/25, 15/26, 15/27, 15/28
BIOT : Keywords as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category*	Citation of Document, [11] with indication, where appropriate of the relevant passages [12]	Relevant to Claim No [13]
X	Molecular Immunology, Vol 25, no 5, pages 429-437 (1988) MALISZEWSKI C.W. et al., "Cloning, sequence and expression of bovine interleukin 1∝ and interleukin 1ß complementary DNAs."	(1, 3, 5-9, 11, 13, 15, 17-29, 31, 33-39)
X	Molecular Immunology, Vol 25, No 9, pages 843-850 (1988) MALISZEWSKI C.W et al., "Bovine GM-CSF: Molecular cloning and billogical activity of the recombinant protein."	(1, 2, 5-10, 13, 14, 17-30, 33-39)
X	Nucleic Acids Research, Vol 18, No 13, page 4012 (1990) McINNES C.J. et al.,"The molecular cloning of the ovine gamma-interferon cDNA using the polymerase chain reaction."	(1, 2, 5-10, 13, 14 17-30, 33-39)

(continued)

* Special categories of cited documents : [10]

"A" Document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior t the international filing date but later than the priority date claimed
"T" Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26 December 1991 (26.12.91)	24 December 91
International Searching Authority	**Signature of Authorized Officer**
AUSTRALIAN PATENT OFFICE	K. AYERS

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	Nucleic Acids Research, Vol 18, No 19, page 5883 (1990) GOODALL J.C. et al., "cDNA cloning of ovine interleukin 2 by PCR."	(1, 3, 5-9, 11, 13, 15, 17-29, 31, 33-39)
P,X	Nucleic Acid Res, Vol 18, No 22, page 6723 (1990) YOUNG A.J. et al., "Primary structure of ovine tumor necrosis factor alpha cDNA."	(1, 3, 4-9, 12, 13, 16-29, 32-39)
P,X	Nucleic Acid Res, Vol 18, No 23, page 7175 (1990) SEOWH.F. et al., "The molecular cloning of ovine interleukin 2 gene by the polymerase chain reaction."	(1, 3, 5-9, 11, 13, 15, 17-29, 31, 33-39)
P,X	Nucleic Acid Res, Vol 18, No 23, page 7165 (1990) FISKERSTRAND C. et al., "Nucleotide sequence of ovine interleukin-1 beta."	(1, 3, 5-9, 11, 13, 15, 17-29, 31, 33-39)

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4a

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.